Assessing the glycaemic and CNS response to sulphonylurea therapy in patients with *KCNJ11* mutations.

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I certify that all material in this thesis which is not my own work has been identified and that any material that has previously been submitted and approved for the award of a degree by this or any other University has been acknowledged.

(Signature) 

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ABSTRACT

ATP-dependent potassium (K_{ATP}) channels are present in the human pancreas, brain, nerve and muscle and play a crucial role in key biological pathways. In the pancreas, K_{ATP} channels regulate insulin secretion from beta cells in response to glucose. They comprise 4 Kir6.2 subunits, encoded by the *KCNJ11* gene, which form the channel pore, and 4 SUR1 subunits, encoded by the *ABCC8* gene, which regulate channel activity and form the binding site for sulphonylurea drugs.

Mutations in K_{ATP} channel genes account for ~half of cases of neonatal diabetes, which is diabetes diagnosed in the first 6 months of life; this may be permanent (PNDM) requiring lifelong treatment, or transient (TNDM) where the diabetes remits and relapses in later childhood or adulthood. Activating mutations in the *KCNJ11* gene are the commonest cause of PNDM. A genetic diagnosis is crucial for patients with these mutations because ~90% can be treated with oral sulphonylureas instead of insulin injections. Sulphonylureas can bind and close mutant K_{ATP} channels allowing endogenous insulin secretion and resulting in improved metabolic control and quality of life at least in the short-term. Severe sulphonylurea-related hypoglycaemia does not occur but mild-moderate episodes, typically related to food, have been reported by affected individuals. The long-term response to sulphonylurea therapy and the regulation of insulin secretion in response to food in people with *KCNJ11* mutations remain key research questions with important clinical implications.

Individuals with *KCNJ11* PNDM can also have central nervous system (CNS) involvement; at its most severe this leads to Developmental delay, Epilepsy and Neonatal Diabetes (DEND) syndrome. Sulphonylurea therapy can also benefit

the neurological features, which are thought to result from the action of sulphonylureas on brain K_{ATP} channels, although the response is only partial in contrast to the excellent glycaemic response. In order to provide appropriate multidisciplinary assessment and support, it is important to establish the specific neurological, psychiatric, and neuropsychological deficits that are present in children and adults with sulphonylurea-treated *KCNJ11* neonatal diabetes, and their impact on affected families.

The overall aim of this thesis is to assess the response to sulphonylurea therapy in patients with PNDM due to mutations in the *KCNJ11* gene, by undertaking clinical studies that investigate the glycaemic response as well as the CNS features in affected individuals.

In chapter 1 we assess the long-term efficacy and safety of sulphonylurea therapy in *KCNJ11* PNDM, by following clinical outcomes relating to both glycaemia and neurological features over 10 years in 81 patients who transferred from insulin to sulphonylureas before December 2006. We show that sulphonylurea therapy is effective and safe long-term, with 93% of individuals remaining on sulphonylureas without adjunctive therapies at most recent follow-up with no reports of severe hypoglycaemia or severe side-effects in over 800 patient years, and normal growth and BMI in children. In addition, we show that neurological features are present in 38/81 individuals and despite initial improvement in 18 individuals on transfer to sulphonylureas, there is persistence of these features to some degree long-term.

In chapter 2 we assess the physiological response to different foods in adults >18 years with sulphonylurea-treated *KCNJ11* PNDM, by measuring glucose, insulin and glucagon levels after a high-protein meal and a high-carbohydrate meal in 5 affected individuals and comparing these with 5 non-diabetic controls.

We show that individuals with sulphonylurea treated *KCNJ11* PNDM have similar insulin levels in response to both a carbohydrate and protein meal despite having higher glucose values in response to a carbohydrate meal than to a protein meal. This contrasts with controls who have higher insulin secretion after carbohydrate than protein and therefore more tightly regulated glucose levels in response to both meals. The findings suggest that individuals with sulphonylurea-treated *KCNJ11* PNDM cannot modulate insulin secretion in response to glucose, consistent with a dependence on non-K_{ATP} pathways for insulin secretion.

In chapter 3 we assess the psychiatric and neuropsychological profile of children <18 years with sulphonylurea-treated *KCNJ11* neonatal diabetes. In study A we use standardised questionnaires to measure psychiatric morbidity and impact in 10 children with *KCNJ11* mutations and compared outcomes with school-age population norms. We show that psychiatric disorders are present in 6/10 children, mainly consisting of autism, attention deficit hyperactivity disorder (ADHD) and anxiety disorders. These disorders are related to the specific mutation (V59M or R201C), have high impact on families and frequently go unrecognised clinically. In study B we use a battery of neuropsychological tests to assess neuropsychological functioning in affected children and compare outcomes to non-diabetic sibling controls. We show that learning difficulties and specific neuropsychological impairments are frequently present even in those children with mutations not consistently associated with a severe CNS phenotype, and that such features are absent from unaffected sibling controls.

In chapter 4 we investigate the neurological, neuropsychological and behavioural features in adults with *KCNJ11* neonatal diabetes, by assessing 8

individuals (7 sulphonylurea-treated) with *KCNJ11* mutations using standardised neuropsychological tests, questionnaires, and clinical history and examination. Outcomes are compared to 4 adults with neonatal diabetes due to mutations in the *INS* gene, thereby controlling for the presence of hyperglycaemia from birth. We show that adults with *KCNJ11* mutations have learning difficulties, features of autism spectrum disorder (ASD), subtle motor dysfunction, moderately reduced IQ, and impaired attention, perceptual reasoning and working memory which persist despite long term sulfonylurea therapy and represent the major burden of disease once glycemia is well controlled on sulphonylureas. The severity of the CNS features varies with the specific mutation and they do not occur in individuals with neonatal diabetes due to *INS* mutations, suggesting they occur as a consequence of dysfunctional brain K_{ATP} channels as opposed to indirect effects of lifelong diabetes.

The conclusions section summarises the data chapters and describes how the work fits together as a coherent whole, as well as identifying directions for future research specific to each of the studies undertaken.

The research in this thesis offers original and novel insights into both the CNS features of *KCNJ11* neonatal diabetes and the glycaemic response to sulphonylurea therapy. Patients with *KCNJ11* neonatal diabetes represent a unique human experimental model for the study of K_{ATP} channel biological pathways in both the pancreas and brain. Treatment of the condition with sulphonylureas remains one of the best examples of precision medicine and illustrates the benefits of targeted treatments in monogenic disease.

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ORGANISATION OF THESIS

Introduction

The introduction is divided into 2 parts. Part 1 gives an overview of neonatal diabetes with a focus on *KCNJ11* mutations as a specific genetic aetiology. It describes genotype phenotype relationships, relevant functional studies and the impact of treatment change from insulin injections to oral sulphonylureas. Part 2 is a review article published in *Journal of Diabetes Research*, entitled 'Future roadmaps for precision medicine applied to diabetes: rising to the challenge of heterogeneity'. This manuscript places sulphonylurea-treated *KCNJ11* neonatal diabetes in the wider context, highlighting it as an outstanding example of precision medicine in diabetes and illustrating the utility of monogenic disorders as human disease models.

Methods

The methods section describes the questionnaires, data collection forms, neuropsychology test batteries and sample handling and analysis procedures used in the research. It focuses on those methods not described in detail within each data chapter and makes reference to the relevant data chapters and Appendix 1 where copies of questionnaires can be found.

Data Chapters

Each data chapter is presented as the final accepted version(s) of one or more peer-reviewed publications. Chapters 1 and 2 focus predominantly on the diabetes caused by *KCNJ11* mutations and chapters 3 and 4 focus on the neurodevelopmental and neurological aspects. Acknowledgements to co-

authors and details of my contribution have been placed at the beginning of each chapter.

Chapter 1

This is an original article published in *Lancet Diabetes and Endocrinology* in 2018, entitled 'Effectiveness and safety of long-term treatment with sulphonylureas in neonatal diabetes due to *KCNJ11* mutations: an international cohort study'. The study is the first long-term follow-up study of glycaemic and neurological outcomes in a large international cohort of 81 patients with *KCNJ11* PNDM over 10 years.

Chapter 2

This is an original article published in *BMJ Open Diabetes Research and Care* in 2019, entitled 'Patterns of post-meal insulin secretion in individuals with sulfonylurea-treated *KCNJ11* neonatal diabetes show predominance of non-K_{ATP}-channel pathways'. The study is the first to assess the physiological response to different types of food in people with *KCNJ11* neonatal diabetes and compare these with non-diabetic controls.

Chapter 3

This chapter is divided into 2 parts, both relating to the neurodevelopmental aspects of *KCNJ11* neonatal diabetes in children. Part A is a short report published in *Diabetic Medicine* in 2016, entitled 'Psychiatric morbidity in children with *KCNJ11* neonatal diabetes'. Part B is a letter published in *Diabetic Medicine* in 2017, entitled 'Neuropsychological impairments in children with *KCNJ11* neonatal diabetes'. These studies use standardised assessments to investigate the psychiatric and neuropsychological profiles of affected children, in comparison to population norms and unaffected sibling controls respectively.

Chapter 4

This is an original article published in *Diabetes Care* in 2018, entitled ‘Cognitive, neurological and behavioral features in adults with *KCNJ11* neonatal diabetes’. It is the first study to assess in detail the neurological, neuropsychological and psychiatric profile of adults with *KCNJ11* neonatal diabetes and to compare these with people with neonatal diabetes due to *INS* mutations.

Conclusions

This is divided into four sections. Each chapter is concluded and the impact of the studies and future directions for research are discussed.

Appendix 1

This appendix contains copies of the documents used for data collection in the studies and any relevant standard operating procedures. These documents are presented in order of the studies in which they were used and referenced in the methods section.

ABBREVIATIONS

ABCC8	ATP Binding Cassette Subfamily C Member 8 (gene)
ACE-R	Addenbrooke's Cognitive Examination-Revised
ADHD	attention deficit hyperactivity disorder
ASD	autism spectrum disorder
AQ	Autism Spectrum Quotient
BBB	blood brain barrier
BB	bio breeding (rat)
BMI	body mass index
CAMHS	child and adolescent mental health services
CBI-R	Cambridge Behavioral Inventory-Revised
cffDNA	cell-free fetal DNA
CHI	congenital hyperinsulinism
CNS	central nervous system
COWAT	Controlled Oral Word Association Test
CPRD	Clinical Practice Research Datalink
CSF	cerebrospinal fluid
CTT	Colour Trails Test
CYP450	cytochrome P450

DAWBA	Development And Wellbeing Assessment
DCCT	Diabetes Control and Complications Trial
DEND	developmental delay, epilepsy and neonatal diabetes
DKA	diabetic ketoacidosis
DKEFS	Delis-Kaplan Executive Function System
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th Edition
EHR	electronic health records
ELISA	enzyme-linked immunosorbent assay
ExAC	Exome Aggregation Consortium
FGM	flash glucose monitoring
GATA6	GATA Binding Protein 6 (gene)
GIP	gastric inhibitory polypeptide
GK	Goto-Kakizaki (rat)
GLP-1	glucagon-like peptide-1
gnomAD	Genome Aggregation Database
GP	general practice
GRS	genetic risk score
GWAS	genome wide association study
HADS	Hospital Anxiety and Depression Scale
HGMD	Human Gene Mutation Database
HNF-1A	Hepatocyte Nuclear Factor 1 Alpha (gene)

HNF-1B	Hepatocyte Nuclear Factor 1 Beta (gene)
iAUC	incremental area under the curve
iDEND	intermediate developmental delay, epilepsy and neonatal diabetes
IEP	individualised education program
IGF	insulin-like growth factor
IGT	impaired glucose tolerance
INS	Insulin (gene)
IQ	intelligence quotient
IQR	interquartile range
K_{ATP}	ATP-sensitive potassium (channel)
KCNJ11	Potassium Channel, Inwardly Rectifying, Subfamily J, Member 11 (gene)
LCMV	lymphocytic choriomeningitis virus (rat)
MRI	magnetic resonance imaging
MODY	maturity onset diabetes of the young
NEPSY	Developmental Neuropsychological Assessment
NOD	non-obese diabetic (mouse)
NSY	Nagoya-Shibata-Yasuda (mouse)
OCD	obsessive compulsive disorder
pmax	maximum serum concentration paracetamol
PNDM	permanent neonatal diabetes mellitus

SD	standard deviation
SDS	standard deviation score
SDQ	Strengths and Difficulties Questionnaire
SEN	special educational needs
SENCO	special educational needs coordinator
SPECT	single photon emission computed tomography
SU	sulphonylurea
SUR1	sulfonylurea receptor 1
tAUC	total area under the curve
tmax	time to maximum serum concentration
TNDM	transient neonatal diabetes mellitus
tNGS	targeted next generation sequencing
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes
VMH	ventromedial hypothalamus
VMI	visual motor integration
WAIS-IV	Wechsler Adult Intelligence Scale, fourth edition
WASI	Wechsler Abbreviated Scale of Intelligence
WISC-IV	Wechsler Intelligence Scale for children, fourth edition
WMS-IV	Wechsler Memory Scale, fourth edition

INTRODUCTION

PART 1

Neonatal diabetes due to mutations in the *KCNJ11* gene: clinical and genetic features and treatment with sulphonylureas

The KCNJ11 (potassium channel, inwardly rectifying, subfamily J, member 11) gene encodes the pore-forming Kir6.2 subunit of the ATP-sensitive potassium channel which is crucial for insulin secretion from pancreatic beta cells

KCNJ11 is a single-exon gene located on the short arm of chromosome 11 (11p15.1) (1) which is expressed in several tissues including brain, muscle, nerves, pancreas and heart (2). Its protein product is Kir6.2, which forms the pore of the ATP-sensitive potassium (K_{ATP}) channel. In the pancreas, K_{ATP} channels are comprised of 4 pore-forming Kir6.2 subunits and 4 regulatory SUR1 subunits, encoded by the *ABCC8* gene (3). This hetero-octameric structure plays a crucial role in glucose-mediated insulin secretion. Under normal physiological conditions, glucose metabolism results in a rise in ATP, which binds to Kir6.2, closing the K_{ATP} channel and preventing efflux of potassium ions across the cell membrane. This results in beta cell membrane depolarisation and influx of calcium through voltage-gated calcium channels. Raised intracellular calcium triggers insulin secretion from the beta cell (4), figure 1.

SUR1 regulates K_{ATP} channel activity by opening the channel in response to MgADP and the binding of drugs such as diazoxide (5). SUR1 also forms the binding site for sulphonylureas, the insulin secretagogues used widely in the treatment of Type 2 diabetes (T2D) which act by closing pancreatic K_{ATP} channels to promote endogenous insulin secretion (6). K_{ATP} channels are also present on alpha cells although a consensus on the specific role of K_{ATP} channel-mediated pathways in glucagon secretion has not yet been reached (7, 8). Furthermore, K_{ATP} channels can be found on hypothalamic glucose sensing neurons in the brain, where they are thought to help drive counter-regulatory responses to hypoglycaemia (9, 10).

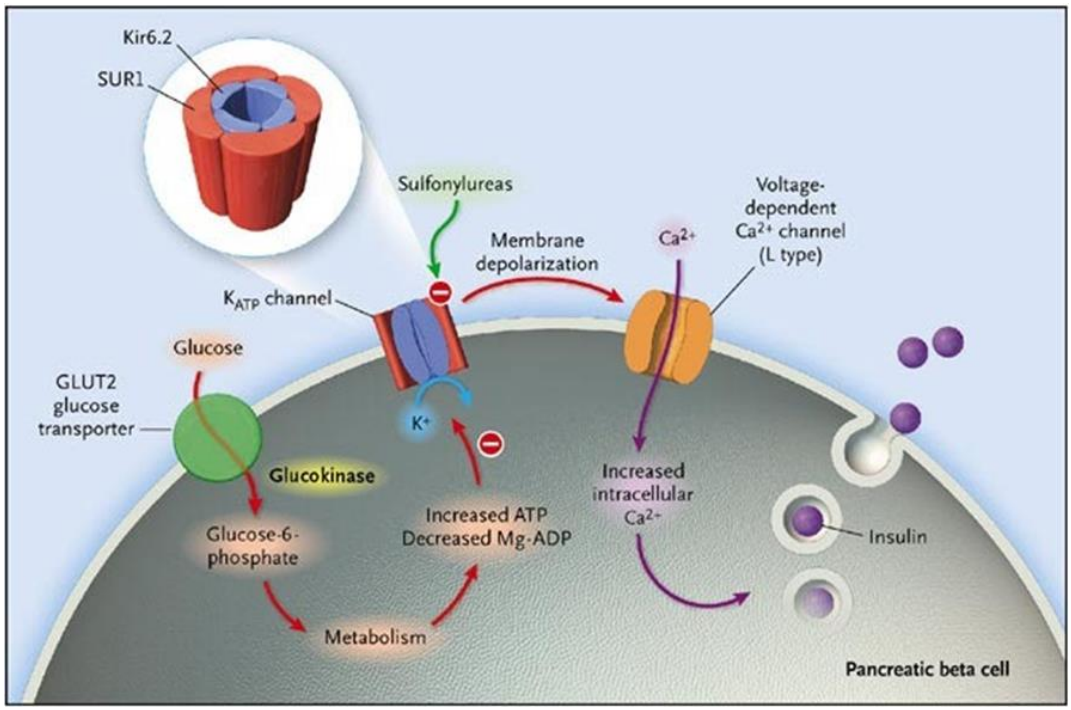

Figure 1, from Gloyn et al. NEJM 2004 (32). Role of the K_{ATP} channel in the pancreatic beta cell in linking blood glucose with insulin secretion.

Dominant activating mutations in KCNJ11 are the commonest cause of permanent neonatal diabetes mellitus (PNDM) and can also cause transient neonatal and adult-onset diabetes

Neonatal diabetes is defined as diabetes occurring in the first 6 months of life and occurs in ~1 in 100,000 live births (11, 12). Nearly half of cases in non-consanguineous populations are caused by mutations in the *KCNJ11* and *ABCC8* genes; *KCNJ11* mutations alone account for ~30% making it the commonest genetic aetiology (13, 14). Most *KCNJ11* mutations arise de novo (~80%) or are dominantly inherited from an affected parent although germline mosaicism resulting in inheritance of disease-causing variants from a clinically unaffected parent has been described (15, 16). These mutations cause neonatal diabetes by rendering K_{ATP} channels insensitive to ATP, affecting gating, increasing the intrinsic open probability or augmenting stimulation of

Kir6.2 by SUR1 (17-20). As a result the channel remains in the open state and unable to respond to rising glucose, which leaves the beta cell permanently hyperpolarised and unable to secrete insulin.

The majority (~80%) of cases of neonatal diabetes due to *KCNJ11* mutations are permanent (PNDM) and persist from diagnosis in infancy throughout life: a smaller number have transient neonatal diabetes, characterised by remission of diabetes at around ~9 months of age with relapse of diabetes in later childhood or adolescence (21, 22). Some *KCNJ11* TNDM mutations exhibit variable penetrance and phenotypic heterogeneity with additional clinical presentations including adult-onset diabetes, childhood-onset diabetes and impaired glucose tolerance (IGT) in pregnancy (23-26). The mechanisms underlying phenomena such as diabetes remission and relapse are not fully understood, and further research is required to address this.

Clinical features of KCNJ11 neonatal diabetes related to the pancreas and CNS

The biological impact of *KCNJ11* mutations begins in utero. Specifically, these mutations reduce birth weight in affected individuals by at least 1 standard deviation (SD) vs the general population (27-29) which is thought to result from insulin deficiency during development leading to reduced insulin-mediated growth (15, 30). Median age at diagnosis of diabetes is ~1 month of age although recent research has shown that glucose is raised in babies with K_{ATP} channel mutations as early as day 5 of life even if they are not diagnosed with diabetes until much later (30). Clinical presentation of *KCNJ11* neonatal diabetes is typically severe; a recent study reported that diabetic ketoacidosis (DKA) was a presenting feature in ~80% of babies with K_{ATP} channel mutations and the odds of this increased with age at diagnosis (14). This severe insulin

deficiency is reflected in the replacement doses of insulin historically required to treat the hyperglycaemia (median insulin dose ~0.7U/kg/day) (28, 29).

In humans, the *KCNJ11* gene is expressed in multiple brain regions but particularly high levels are found in the cerebellum (2). Mutations in *KCNJ11* can therefore cause central nervous system (CNS) features in addition to diabetes. Early studies showed that around one fifth of patients had a severe CNS phenotype comprising developmental delay (usually global and severe in nature), epilepsy and neonatal diabetes, the so-called DEND syndrome (27, 28, 31). Intermediate DEND (iDEND) was the term used if there was no epilepsy in the first 12 months of life; typically, these patients had developmental delay in the moderate-severe range (15, 27, 32). Muscle weakness, impaired coordination, visuomotor performance, hand-eye tracking and features of autism spectrum disorder (ASD) were also reported in individuals with DEND / iDEND syndrome (31, 33-36). More recently, research studies have shown that the CNS features in *KCNJ11* PNDM extend beyond the severe overt syndromic phenotype. In one cohort study of 27 patients without DEND / iDEND, the majority (almost 80%) were shown to have dyspraxia and / or attention difficulties when assessed using standardised neuropsychological tests (22). This was supported by research showing high rates of neuropsychological impairments in children with *KCNJ11* mutations who did not have severe developmental delay when compared to sibling controls without diabetes (37).

Impact of glycaemic factors on CNS phenotype

Glycaemic factors can have neurological sequelae and it is important to consider the potential impact these may have in the context of neonatal diabetes, and how they are different to the CNS features that occur directly as a result of the genetic mutation. The severe DEND / iDEND syndrome is typically

associated with muscle weakness (32). This is thought to occur as a direct result of dysfunctional brain K_{ATP} channels as selective expression of the iDEND-associated V59M mutation in rat brain (but not muscle or peripheral nerves) replicates the human phenotype (38). In contrast, cerebral oedema due to diabetic ketoacidosis (DKA) at diagnosis causes spastic tetraplegia (39). However, as the majority of children with *KCNJ11* mutations present in DKA (see above), they are also at significant risk of severe neurological impairment secondary to the metabolic disturbance associated with this acute severe presentation.

Furthermore, having diabetes from childhood is associated with a range of subtle cognitive impairments. A meta-analysis of children with Type 1 Diabetes (T1D) showed mild-moderate impairments (in order of decreasing effect size) in intelligence, psychomotor efficiency, cognitive flexibility, visual perception, attention, and processing speed, when compared to children without diabetes (40). Cognitive deficits were more pronounced in those diagnosed with diabetes early in life i.e. between the ages of 4-7 years, and in those with microvascular complications of diabetes (40, 41). Interestingly recurrent severe hypoglycemia did not have an effect on cognitive performance (40). Learning and memory appear to be relatively spared in children and younger adults with diabetes in contrast to older adults (>60-65 years) with Type 2 Diabetes (T2D) in whom impairments in these cognitive domains are the most predominant (42). Neuroimaging studies in T1D have demonstrated alterations in grey and white matter volume in several brain regions in comparison to non-diabetic controls; typically, there is a negative correlation with HbA1c and disease duration (43).

Such studies should be taken into consideration in the context of the constellation of neuropsychological features observed in *KCNJ11* neonatal diabetes. Although the evidence to date suggests that the CNS profile in individuals with neonatal diabetes is largely a direct result of the dysfunctional K_{ATP} channels in the brain (44), the extent to which other diabetes-related factors play a role has not been fully elucidated. There are several mechanistic theories as to how states of hyper- or hypoglycaemia may impact on the brain, particularly during periods of rapid brain development in childhood. These include hyperglycaemia-induced oxidative stress and neurodegradation and hypoglycaemia-related energy deprivation resulting in cell death (43). Disentangling these effects from those occurring directly as a result of K_{ATP} channel mutations represents an interesting aspect of future research.

Further exploration of the cognitive profile in children with sulphonylurea-treated *KCNJ11* neonatal diabetes is described in Chapter 3 of this thesis. The question of whether diabetes per se from a very early age affects CNS function long-term in affected individuals is addressed in chapter 4, which compares the CNS features in adults with sulphonylurea-treated *KCNJ11* neonatal diabetes to adults with insulin-dependent diabetes from birth due to mutations in the *INS* gene.

Genotype phenotype relationships and functional studies

In individuals with *KCNJ11* mutations certain phenotypes are more commonly associated with specific mutations. For example, neurological features are frequently seen in patients with the V59M mutation, PNDM without overt CNS features in patients with the R201H mutation, and TNDM in those with the E227K mutation (21, 28). It has been suggested that one reason for this relates to the position of the affected amino acid in the Kir6.2 protein structure and the

functional effect(s) of this on the K_{ATP} channel (27). The R201 residue and others causing neonatal diabetes without severe neurological involvement lie within the ATP binding site and therefore directly affect ATP sensitivity, whereas V59M and others causing DEND/iDEND syndrome are located more distantly from the ATP binding site and may have additional functional effects such as affecting channel gating and open probability (19, 27). Functional studies have shown that there is some association between the severity of the clinical phenotype and the severity of the mutation *in vitro*, at least for variants known to cause DEND/iDEND (18, 28). Those mutations associated with significant neurological symptoms tend to have larger resting whole cell K_{ATP} channel currents than those associated with PNDM or TNDM (~28-40% vs ~7-10%) (17, 19, 45).

No clear functional associations have been established in relation to diabetes phenotype in the absence of overt CNS features, with no difference in the size of K_{ATP} channel currents in the presence of variants that cause PNDM, TNDM or both (17). In addition, although broadly speaking some genotype-phenotype relationships such as those described above hold true, there are notable exceptions e.g. a few cases of non-syndromic *KCNJ11* PNDM have been reported in association with V59M, and PNDM or later onset diabetes in association with E227K (27, 46, 47). This emphasises the roles of other genetic and environmental factors in determining the phenotypic manifestations of specific *KCNJ11* mutations.

Neonatal diabetes due to KCNJ11 mutations can be treated with oral sulphonylureas: an outstanding example of precision medicine

Early functional studies showed that *KCNJ11* mutations did not prevent inhibition of K_{ATP} channels by sulphonylureas (17), suggesting that these drugs may also be a potential treatment for patients with *KCNJ11* neonatal diabetes. In-keeping with this, physiological studies in small numbers of affected individuals demonstrated insulin secretion in response to intravenous (IV) tolbutamide but not IV glucose (32). Subsequently, oral sulphonylurea therapy was shown to be effective in 4 affected patients over 2-6 months allowing discontinuation of insulin (48, 49). Glibenclamide treatment was associated with improved (48, 49) or equivalent (48) glycaemic control in comparison to that observed on insulin therapy, as well as a reduction in glycaemic variability measured using continuous glucose monitoring (CGM) (49). The first large cohort study to investigate the treatment of *KCNJ11* neonatal diabetes with sulphonylureas showed 44/49 patients successfully transferred and had excellent outcomes with median HbA1c falling from 8.1% on insulin to 6.4% 12 weeks after switching to sulphonylureas (29). This improvement was maintained at 1 year and not associated with any severe side-effects or increased hypoglycaemia despite the need for large doses (median 0.45mg/kg/day glibenclamide) (29).

Further research has shown that the main factors affecting ability to transfer successfully are the specific genetic variant and the age at transfer (50, 51). The former relates to the effect of the mutation on in vitro tolbutamide block; one study of 127 individuals showed that those who had mutations with a tolbutamide block of >73% were able to transfer in the majority of cases (50). The minority who did not transfer were older when the switch was attempted (50). This is in-keeping with a study of 58 individuals which reported a need for additional medication to maintain glycaemic control in 10/17 patients who were

older than 13 years at the time of transfer, with those under 13 years consistently able to manage on sulphonylurea monotherapy (51). One reason for this may relate to more marked changes in beta cell gene expression and function with prolonged exposure to hyperglycaemia on insulin therapy. This hypothesis is supported by a mouse model of *KCNJ11* neonatal diabetes in which selective expression of the V59M mutation in pancreatic beta cells leads to development of diabetes and changes in islet morphology, structure and gene expression after just 4 weeks (52). Interestingly those mice who were given glibenclamide after 2 days of diabetes require lower doses of the drug than those who were treated after 4 weeks (52). Taken together, these studies indicate that earlier sulphonylurea transfer increases the chances of success and support rapid early genetic diagnosis.

In the large number of individuals with *KCNJ11* mutations who are able to successfully transfer from insulin to sulphonylureas, there is a significant psychological impact for both the patients themselves and their families. Parents of babies and young children with *KCNJ11* neonatal diabetes described moving from daily states of anxiety and fear of separation from their child whilst on insulin treatment, to greatly improved quality of life, more freedom and less distress following transfer of their child to sulphonylureas (49, 53). This was largely due to the improved metabolic control and reduction in glycaemic variability and thus diminished need for vigilance for symptoms of hypoglycaemia (53-55). Some adults who had been insulin-treated since diagnosis viewed insulin as part of their identity; they were initially apprehensive about the prospect of changing treatment and required a period of adjustment to become accustomed to this (53, 54, 56). Despite this, the psychological impact of treatment change, when complete, was largely extremely positive (56). Even

in the few cases unable to transfer from insulin to sulphonylureas, a genetic diagnosis was helpful particularly in those cases with overt CNS features in addition to diabetes. This is because identification of a genetic aetiology offered an explanation for the presence of these features together; previously they were thought to be unrelated (57).

Sulphonylureas fail in T2D but mechanism of action is likely to be different in NDM given different patterns of hypoglycaemia and tolerance of large doses

In T2D additional treatment is required in between one third and one half of cases after 5-6 years of monotherapy with sulphonylureas to maintain adequate glycaemic control (58, 59). Studies in small cohorts of patients with *KCNJ11* PNDM suggested that the improved glycaemic response following transfer to sulphonylureas lasted for at least 3-5 years (60, 61), but prior to the research in this thesis, the long-term effects remained unknown with questions around the safety of high doses, especially in children. In T2D a known side-effect of sulphonylurea therapy is hypoglycaemia, which can be severe in nature and typically occurs in the fasting state (62, 63). Patients with *KCNJ11* PNDM can tolerate much higher doses of sulphonylureas than patients with T2D without experiencing severe hypoglycaemia (29). Furthermore, any mild-moderate hypoglycaemia that occurs in sulphonylurea-treated *KCNJ11* PNDM may be related to meals, a different pattern to that observed in T2D (51, 64). Such observations suggest that the mechanism of action of sulphonylureas in patients with *KCNJ11* mutations is also different to that in T2D. Early physiological studies showed that individuals with *KCNJ11* mutations on sulphonylurea therapy could secrete insulin in response to glucose given orally but not intravenously, implying that the presence of food was essential for this process either through GLP-1 or nutrient stimulation of beta cells (29). It was

suggested that the action of sulphonylureas in *KCNJ11* PNDM was therefore permissive in nature, allowing the beta cell to respond to amplifying pathways of insulin secretion (29). This contrasts with the situation in T2D where sulphonylureas directly inhibit K_{ATP} channels to cause beta cell depolarisation and drive endogenous insulin secretion (65).

Chapter 1 of this thesis addresses the crucial question of the long-term durability of sulphonylurea therapy in *KCNJ11* PNDM including drug efficacy and safety over 10 years. Chapter 2 explores the mechanistic questions relating to insulin secretion in more detail by measuring the physiological response to different foods in patients with sulphonylurea-treated *KCNJ11* neonatal diabetes. It demonstrates the utilisation of amplifying non K_{ATP}-channel-mediated pathways of insulin secretion, which predominate over the classical ATP pathway in contrast to the situation in the non-diabetic state where individuals remain glucose-responsive.

CNS features improve following treatment with oral sulphonylureas

An unexpected benefit of treating *KCNJ11* neonatal diabetes with sulphonylureas was an improvement in the CNS features associated with the condition, with early case reports and clinical anecdotes describing changes in motor function, IQ and attention that occurred fairly soon after sulphonylurea transfer (35, 57, 66, 67). It was suggested that this was due to a central action of sulphonylurea therapy in the brain as opposed to simply improved diabetes control. In-keeping with this, single photon emission computed tomography (SPECT) scanning showed enhanced cerebellar perfusion in 4/5 individuals 6 months after insulin to sulphonylurea transfer when compared with pre-transfer perfusion. In one individual, this was also associated with resolution of a temporal lobe perfusion defect and significant clinical improvement in

neurological and cognitive functioning (45, 67). Further neuroimaging studies in 17 individuals with sulphonylurea-treated *KCNJ11* neonatal diabetes showed no significant structural abnormalities of the brain, but white matter hyperintensities in 10 patients, consistent with what had been reported in a previous case (32, 44).

Although the clinical improvements in neurological functioning were an exciting finding in individuals who transferred to sulphonylureas, it became evident that they were often incomplete, and patients were frequently left with residual deficits. The only study to examine this prospectively showed that after 12 months of sulphonylurea therapy, in children <4 years of age there was marked improvement in fine and gross motor skills, tone and attention (44). In those >4 years of age, there was improvement in tone, laterality, visual attention and visuospatial integration, but the changes were not as marked as those observed in the younger children (44). This supports previous research that demonstrated a correlation between the age of initiation of sulphonylurea therapy and visuomotor performance in patients with iDEND-related mutations (34). A more recent study has questioned this hypothesis by finding no effect of earlier sulphonylurea treatment on neurodevelopmental outcomes in patients with the V59M mutation; however the analysis only included 5 individuals and may not have been sufficiently powered to detect differences (68). Research in other fields suggests that there may be a 'window' of ~6 months following birth whereby interventions may have the most impact on CNS function, which is thought to be related to increased neuroplasticity of the young brain (69-71). Currently clinicians are encouraged to start sulphonylurea therapy in patients with *KCNJ11* neonatal diabetes as early as possible, but further research in larger cohorts of patients is required to provide a definitive answer as to

whether the early initiation of treatment (in the first 6 months of life) brings measurable improvements in neurodevelopmental outcomes over starting treatment later in life.

Another hypothesis as to why there is only partial recovery of the CNS with sulphonylurea treatment is that insufficient drug levels are achieved in the cerebrospinal fluid (CSF). In rats, extremely high systemic doses of glibenclamide are required to obtain measurable levels in the CSF, and the drug is rapidly exported back out of the brain via an active transport mechanism as demonstrated by rapidly diminishing CSF concentrations following intraventricular administration (72). The extent to which this also occurs in humans has not been established, although anecdotally patients with significant neurological involvement report greater improvements on doses of ~1mg/kg/day glibenclamide or occasionally even higher (44, 73). Similarly, the CNS penetration and clinical effects on neurological function of sulphonylureas other than glibenclamide has not been studied and this is an area where further research will be crucial in the future, to inform clinical recommendations for patients.

Finally, most of the studies described previously have identified neuropsychological impairments after transfer to sulphonylureas and it is not clear whether larger doses would benefit these more subtle impairments. Interestingly the one patient who did not show improvement in cerebellar perfusion on transfer to sulphonylureas in the study by Fendler et al carried the R201H mutation which is regarded as clinically milder given its lack of association with overt neurological features (45). Further studies are required to investigate the impact of treatment-related factors on patients with only subtle CNS features.

Despite these caveats, the positive impact of sulphonylurea therapy on the neurobehavioural features in many patients with *KCNJ11* mutations is very exciting. Not only does it present an opportunity to improve CNS function in affected children, but also it highlights the possibility of precision therapy for developmental disorders related to monogenic disease more broadly. This will be a key area for research in the future.

The long-term neurological outcomes in patients with sulphonylurea-treated *KCNJ11* neonatal diabetes is explored in Chapter 1 of this thesis. In addition, chapters 3 and 4 give a more detailed account of the CNS features that persist in affected children and adults.

ABCC8 neonatal diabetes shares many features in common with KCNJ11 neonatal diabetes but requires further study as a separate clinical syndrome

Mutations in the *ABCC8* gene, which encodes the SUR1 subunit of the K_{ATP} channel, account for ~15-20% cases of neonatal diabetes in non-consanguineous populations, making this the second commonest known cause after *KCNJ11* mutations (13). SUR1 regulates K_{ATP} channel activity by MgADP (74) and is the binding site for channel inhibitors and openers such as sulfonylureas and diazoxide (74, 75). Although the molecular effects of *ABCC8* mutations are distinct from those of *KCNJ11* mutations, the cellular effects are similar, with reduced sensitivity to ATP-mediated channel closure, beta cell hyperpolarisation and lack of insulin secretion (76).

Initial descriptions indicated that the clinical features of *KCNJ11* and *ABCC8* neonatal diabetes were similar; specifically, there were no significant differences in birth weight, presence of ketoacidosis, or age at diagnosis of diabetes (76). However, there are some notable differences both clinically and

genetically. Whilst *KCNJ11* mutations result in PNDM in the majority (~80%) cases, *ABCC8* mutations frequently cause TNDM, with PNDM occurring in only ~20% cases (22). Variants in the *ABCC8* gene causing neonatal diabetes tend to be a mixture of dominant heterozygous mutations as well as compound heterozygous, homozygous or mosaic variants (77), whereas dominant activating mutations predominate in *KCNJ11* neonatal diabetes. In *ABCC8* neonatal diabetes genotype-phenotype relationships are not particularly distinct, in contrast to *KCNJ11* neonatal diabetes where the correlation is stronger as described above (78). Finally, there is no animal model of *ABCC8* neonatal diabetes unlike the V59M mouse, which has had some utility in generating hypotheses about human neonatal diabetes due to *KCNJ11* mutations (38, 52, 79). Some or all of these factors may explain why, broadly speaking, neonatal diabetes due to *ABCC8* mutations has not been so extensively researched as *KCNJ11* neonatal diabetes.

Nevertheless, *ABCC8* neonatal diabetes is amenable to treatment with sulphonylureas with ~85% patients able to transfer from insulin onto glibenclamide with improvements in glycaemic control, which is maintained in the short-term (80). Previous research has suggested that lower doses of sulphonylurea are required to treat *ABCC8* neonatal diabetes in comparison to *KCNJ11* neonatal diabetes (80, 81), although many studies of *ABCC8* neonatal diabetes contained a higher proportion of individuals with TNDM (76, 80) which may have resulted in a lower average dose. Further studies are needed specifically in *ABCC8* PNDM to assess this in more detail.

ABCC8 and *KCNJ11* are both expressed in the brain as well as the pancreas (82, 83), which explains why neurological features have also been described in patients with *ABCC8* mutations. Early observational studies suggested that the

CNS phenotype may be less frequent and / or severe in *ABCC8* neonatal diabetes than in individuals with *KCNJ11* mutations (76, 84, 85), although detailed neurobehavioural assessments of *ABCC8* patients are lacking. In the largest cohort study to date of individuals without DEND/iDEND, subtle deficits in attention and praxis were present in a similarly high proportion (~80-100%) of *KCNJ11* and *ABCC8* patients (22). In addition, the more overt CNS features appear to respond to a degree to sulphonylurea therapy. In 2 patients with *ABCC8* mutations followed prospectively in the study by Beltrand et al., both had improved tone after 12 months of treatment with sulphonylureas, although other neurological features persisted (44). Further investigation of the CNS features and response to treatment specifically in patients with *ABCC8* neonatal diabetes will be crucial to allow more accurate genetic counselling and clinical management of this subgroup of patients in the future.

Impact of technology on diagnosis and management in neonatal diabetes

The replacement of Sanger sequencing with high throughput targeted next generation sequencing (tNGS) means that many genes can now be tested simultaneously, rapidly and relatively cheaply (86). Gene panels used to test for neonatal diabetes yield a genetic diagnosis in over 80% of cases (13). In non-consanguineous populations, almost half of the cases identified will have a K_{ATP} channel mutation and will be sulphonylurea responsive therefore a rapid genetic diagnosis is crucial for selecting the optimum treatment. Furthermore, early genetic diagnosis facilitates prediction of prognosis including development of extra-pancreatic features (13).

As cohorts of patients with *KCNJ11* neonatal diabetes become older, the number of monogenic pregnancies will rise and in each case, there will be a 50% chance of the offspring of an affected parent inheriting the causal variant.

The use of cell free fetal DNA (cffDNA) to identify those babies who have inherited mutations at an early stage of development (87) affords opportunities at both a clinical and research level. Glibenclamide can cross the human placenta and stimulate the fetal pancreas (88). This is supported by reports of macrosomia due to excess insulin-mediated growth and neonatal hypoglycaemia in non-diabetic infants of mothers with *KCNJ11* mutations who have been treated with sulphonylureas during pregnancy (89). However, for the affected fetus of a mother with a *KCNJ11* mutation the potential benefits of maternal glibenclamide therapy in pregnancy are two-fold. Firstly, stimulation of fetal insulin secretion can normalise birth weight (90). Secondly, there is a potential positive impact on neurodevelopment in the affected fetus, although this would have to be tested formally in a longitudinal research study. Therefore, cffDNA is another exciting technological advance that is making extremely early diagnosis, selection of specific therapies in pregnancy and prospective research into the *in utero* effects of *KCNJ11* mutations a possibility for affected individuals.

Conclusions and contributions of this thesis to the scientific literature

Discovery of the *KCNJ11* gene and its role in neonatal diabetes was a hugely exciting breakthrough that changed many lives for the better by allowing treatment change from insulin to sulphonylureas. Affected individuals not only exemplify beautifully the utility of precision medicine in monogenic disease, but they represent unique human models for the study of biological pathways related to K_{ATP} channels which may have wider relevance for more common polygenic conditions. Research in the field has grown rapidly over the past 15 years, with increasing interest in the CNS manifestations of *KCNJ11* mutations

as well as the diabetes and the response of both aspects to sulphonylurea therapy.

The research described in this thesis addresses key gaps in knowledge relating to *KCNJ11* neonatal diabetes, by investigating crucial questions about sulphonylurea treatment response and exploring both pancreatic and extra-pancreatic features of the condition. Part 2 of the introduction and all of the data chapters are peer-reviewed publications, which have made a significant contribution to the scientific literature, as well as forming the basis for future studies in the field of neonatal diabetes. Importantly, the translational nature of the research has also resulted in a positive impact on patient care.

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INTRODUCTION

PART 2

Future roadmaps for precision medicine applied to diabetes: rising to the challenge of heterogeneity

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Abstract

Precision medicine, the concept that specific treatments can be targeted to groups of individuals with specific genetic, cellular or molecular features, is a key aspect of modern healthcare and its use is rapidly expanding. In diabetes the application of precision medicine has been demonstrated in monogenic disease, where sulphonylureas are used to treat patients with neonatal diabetes due to mutations in ATP-dependent potassium (K_{ATP}) channel genes. However, diabetes is highly heterogeneous, both between and within polygenic and monogenic subtypes. Making the correct diagnosis and using the correct treatment from diagnosis can be challenging for clinicians, but it is crucial to prevent long-term morbidity and mortality. To facilitate precision medicine in diabetes, research is needed to develop a better understanding of disease heterogeneity and its impact on potential treatments for specific subtypes. Animal models have been used in diabetes research but they are not translatable to humans in the majority of cases. Advances in molecular genetics and functional laboratory techniques, and availability and sharing of large population data provide exciting opportunities for human studies. This

review will map the key elements of future diabetes research in humans and its potential for clinical translation to promote precision medicine in all diabetes subtypes.

Introduction

Diabetes is a heterogeneous group of metabolic disorders that represents an enormous health burden globally. In 2014, an estimated 422 million adults had diabetes, and the prevalence continues to rise.[1,2] Complications related to diabetes cause significant morbidity and mortality.[1] At a time when healthcare resources to support an ageing population are limited, it is crucial to develop more effective treatments and make sure that patients receive the treatment appropriate to their condition.

Diabetes is multifactorial and caused by both genetic and environmental factors. Monogenic forms of diabetes (caused by mutations in single genes), including Maturity Onset Diabetes of the Young (MODY) and neonatal diabetes (diagnosed before 6 months of age), are rare, representing ~3.6% of all cases diagnosed under 30 years.[3] Indeed, for most types of diabetes, multiple genes are involved. Type 1 diabetes (T1D) is characterised by insulin deficiency most often resulting from immune-mediated destruction of pancreatic beta (β) cells, whilst Type 2 diabetes (T2D) results from insulin resistance and hence β cell failure.[4] Also, it is becoming clear that specific subtypes within T1D and T2D have different aetiologies. Correct diagnosis is crucial to allow selection of appropriate therapy, but this can be a challenge for clinicians; even the UK Prime Minister was misdiagnosed as having T2D and started on the wrong treatment before it became apparent that she had T1D requiring insulin

therapy.[5] Indeed, up to 15% of patients with diabetes are misclassified in Primary Care in England.[6] A recent cross-sectional study showed rates of misclassification are particularly high in those patients with T2D (defined by presence of significant endogenous insulin secretion more than three years after diagnosis) who are older (>34 years) at diagnosis and who start insulin immediately; they are misclassified as T1D in around half of cases.[7] This experience is not unique to the UK; an 11 year follow-up of an American paediatric diabetes cohort revealed initial misclassification of diabetes in over 20% of individuals.[8] Add to this the heterogeneity within T1D and T2D, and diagnostics and treatment become a major challenge even for the most experienced clinicians.

Precision medicine is the tailoring of treatment to specific molecular or cellular characteristics of groups of patients; this can also be influenced by environmental and lifestyle factors. Precision medicine is rapidly becoming a key concept in many areas of modern clinical practice.[9] Perhaps its most widely recognised application is in oncology, where the specific genetic profile of the tumour can determine the targeted treatment.[10,11] However, the precision approach has also been applied to other areas of medicine[9], and the field is rapidly developing, largely due to ongoing advances in molecular genetic techniques such as next generation sequencing (NGS).[12]

There is increasing interest in applying precision medicine to diabetes. In fact, it has already been done in rare monogenic subtypes of the disease, but there are challenges when it comes to applying precision medicine to T1D and T2D.[13-15] One aspect that makes translational research for new targeted treatments particularly challenging in common polygenic subtypes of diabetes is the heterogeneity within these broad disease categories as described above.

The first step will be developing an understanding of the often subtle differences in pathophysiology and factors influencing treatment response between individuals with the same ‘type’ of diabetes. This has not been possible using artificial animal models of diabetes. Even though the recent advances in human research methods are more promising than using animals, still some difficulties exist as the lessons learned from monogenic disease are not readily translatable to polygenic diabetes.[13]

To facilitate application of a precision medicine approach in diabetes, a comprehensive map of the pathophysiology and treatment targets for each known diabetes subtype is needed, in-keeping with the ‘adverse outcome pathway’ based approach applied to human drug discovery.[16] In this review, we will outline why applying precision medicine to diabetes is unachievable using research findings from traditional animal models, and discuss the challenges faced in future translational research in the field.

Animal models of diabetes are not reliably translatable to humans

Animals have been used in diabetes research for over a century in an attempt to create models that are relevant to humans.[17,18] To date, there is no single animal model that accurately represents all aspects of human T1D or T2D. Rodent models have provided some insights into isolated pathways and mechanisms relevant to polygenic diabetes without the time and expense associated with clinical trials and long term follow up studies in humans.[19] However, results from these experiments must be interpreted with caution. Most animal models have little relevance to human diabetes; this is exemplified by

the problems encountered when attempting to translate animal models of T1D and T2D to humans.

Animal models in T1D

Early spontaneous rodent models of T1D include the Non Obese Diabetic (NOD) mouse 20 and the bio breeding (BB) rat.[21-23] In the NOD mouse, insulitis occurs at 3-4 weeks of age and is accompanied by infiltration of islets by CD4 and CD8 lymphocytes, resulting in cytotoxicity and β-cell destruction with onset of overt diabetes at around 18 weeks. However, the patterns of insulitis seen in NOD mice are different to those observed in human T1D.[24] In addition, there are significant gender differences in the prevalence of diabetes in NOD mice,[25] with females showing earlier onset and more aggressive disease, likely due to modification of cytokine production and *STAT4* gene expression by sex hormones.[25] The gender difference noted in the mice is not apparent in human T1D; this is one of the few circumstances where an autoimmune disease does not occur more frequently in females.[26] BB rats develop diabetes at 8-16 weeks of age and have severe insulin deficiency, but despite not showing the gender differences seen in NOD mice, the rats are lymphopaenic [27], which is not a characteristic of T1D in humans. Importantly, in both NOD mice and BB rats, therapeutic interventions for diabetes that have shown promise e.g. oral insulin and nicotinamide, have not been successful when tried in humans.[28,29] More recently, the Akita mouse, which has a mutation in the insulin 2 gene, has been used as a genetically-induced model of T1D.[30] These mice show severe insulin deficiency and have a short lifespan; however, caution should be used in extrapolating findings from a monogenic model in a rodent to a more complex polygenic disease in humans, as the

pathophysiologies are likely to be different. The lymphocytic choriomeningitis virus (LCMV) rat is a virus-induced model of T1D.[31] LCMV is a rodent-borne virus but if human infection occurs, there can be neurological sequelae, particularly in the context of congenital infection.[32] However, LCMV has not been linked with diabetes in humans. Indeed, the types of viruses and their precise role in the pathophysiology of T1D in humans is still an active area of research,[33] therefore the mechanisms of disease are likely to be different to the LCMV-induced rat model.

Animal models in T2D

Rodent models of T2D can be categorised into obese and non-obese and are similarly flawed by their inability to fully capture the human phenotype. Non-obese models, generated by selective inbreeding, include the Nagoya-Shibata-Yasuda (NSY) mouse and the Goto-Kakizaki (GK) rat.[34,35] Similar to the T1D NOD mouse, the NSY mouse shows gender differences in the prevalence of diabetes[25], with a cumulative incidence of diabetes of 98% and 31% at 48 weeks of age in males and females respectively.[34] This pronounced male excess is not observed in humans with T2D.[26] GK rats have had some utility in the study of diabetes complications and beta cell dysfunction but limitations include significant heterogeneity between different rodent populations leading to variation in the aetiology of hyperglycaemia, which appears to be mainly due to beta cell dysfunction and / or reduced mass as opposed to insulin resistance.[18]

The most widely used models of T2D in animals are the obese models, comprising the monogenic leptin-deficient ob/ob mouse and the leptin receptor

deficient db/db mouse.[36,37] Both have severe obesity as well as hyperinsulinemic hyperglycemia.[18] In humans, it is known that the monogenic leptin deficiency from either a leptin or a leptin receptor gene mutation is associated with unregulated appetite and very severe obesity[38,39], but despite this, T2D has not been described to date in these patients. The most severe hyperglycaemia in ob/ob mice occurs aged 3-5 months and the severity decreases thereafter; islet volume in the pancreas is increased and insulin secretion is maintained.[40] This process does not reflect the β cell failure seen in human T2D. In db/db mice, ketosis occurs at a few months of age and they do not live long (only 8-10 months).[41] Again, this does not reflect the natural history of T2D in humans. For full detailed reviews of animal models in diabetes, see King A, British Journal of Pharmacology 2012.[18]

Animal models in monogenic diabetes

Rodent models of monogenic diabetes have tended to follow on from the discoveries of single gene aetiologies in humans. They have had some utility in providing support for hypotheses relating to mechanism and expression patterns for specific genes, particularly in MODY caused by mutations in the transcription factors Hepatocyte Nuclear Factor 1 Alpha (*HNF-1A*)[42,43], Hepatocyte Nuclear Factor 1 Beta (*HNF-1B*)[44], and in neonatal diabetes due to *KCNJ11* mutations.[45-47]. However, the phenotype of the monogenic mouse, both in relation to diabetes and extra-pancreatic features, is not always consistent with what is observed in humans.[48,49] In addition, the natural history of disease may differ, for example humans with glucokinase MODY do not have renal complications long-term which contrasts with the proteinuria and structural kidney changes observed in a liver-specific hemizygous glucokinase

knockout mouse model.[50,51] These issues limit translatability of such animal models to monogenic diabetes in humans.

Human research is needed to address the questions that cannot be answered using animals

The fundamental differences in the natural history of T1D and T2D in animal models and humans makes it impossible to interrogate these broad disease categories at an individual or indeed subgroup level using rodents. Monogenic diabetes rodent models bear a slightly closer resemblance to their human equivalents but clinical translation remains limited. As research in animals does not provide the insights into the heterogeneity of diabetes that are needed for therapeutic advances in the field, new approaches, focusing on research in humans, are needed (Table 1).

Advances in human molecular genetics have driven treatment change and improved clinical care in monogenic diabetes

We have outlined the significant limitations of using a monogenic disease in animals to model a disease that is polygenic in humans. However, one key question is whether we can learn lessons from monogenic diabetes in humans that are generalisable to polygenic forms of diabetes.

Models in diabetes research		Utility	Limitations	Facilitators	Future potential
Human	Populations	* GWAS for risk variants in polygenic disease and new gene discovery studies for monogenic disease * Risk and treatment stratification using biomarkers and clinical features * Clinical trials of new / repurposed treatments	* Large-scale bioinformatics support and data management / storage required with cost implications * Ethical implications of use and long-term storage of genetic data * Functional and clinical interpretation of genetic data is challenging particularly if vast quantities	* High throughput genomic sequencing techniques e.g. NGS * Data sharing via human gene / disease / clinical databases, clinical trial data access * Integration of research into clinical practice e.g. 100,000 genomes project * Electronic Health Records	+++
	Beta cells	* Mapping pathways and regulatory networks in combination with molecular genetic data * Determining role of immunological / environmental factors	* Difficult to obtain large numbers of specimens from cadaveric donors * Does not capture multi-system physiology and so may not be fully translatable to whole organism	* High throughput genomic sequencing techniques e.g. NGS * Improved interpretation of GWAS findings * Advances in laboratory techniques	++
Animal	Induced	* Can provide some supporting evidence of disease causality or association for genetic / environmental factor(s) being studied	* Differences in aetiology and natural history of disease between animals and humans limits clinical translation / utility * Not useful for testing therapeutic interventions as differences in animal and human responses	* Advances in molecular genetic techniques including genetic manipulation	-/+
	Spontaneous	* May help generate hypotheses about factors involved in disease aetiology / pathophysiology			-

Table 1. Opportunities and limitations in diabetes research.
NGS = next generation sequencing.
+++ = excellent potential for future advances
++ = good potential
+ = possible potential
- = limited potential

Advances in human genetics have revolutionised monogenic diabetes research and clinical care for affected families by accelerating gene discovery and

allowing better treatments to be developed for some subtypes. Historically, single candidate genes for a disease in question were screened using Sanger sequencing. This is an accurate method of sequencing, but the analysis is relatively slow and expensive as single genes need to be analysed sequentially in sections (by exon). Sanger sequencing of specific genes is therefore not ideal for disorders where there is significant overlap in phenotype both within and between different genetic aetiologies, or where the genetic cause is not yet known. Next-generation sequencing is a relatively new technique that allows sequencing of many genes all at once, at a similar cost to sequencing just a few genes by the traditional Sanger method.[12] This is highly advantageous in monogenic diabetes, where an early and rapid genetic diagnosis is crucial for two reasons. Firstly, there are treatments that are available for specific types of diabetes but not for others. For example, Maturity Onset Diabetes of the Young due to *HNF1A/4A* mutations can be treated with low-dose sulphonylureas; neonatal diabetes due to potassium channel gene mutations can be treated with high-dose sulphonylureas, whereas mild fasting hyperglycaemia due to glucokinase mutations does not require pharmacological treatment.[13] Secondly, early identification of diabetes caused by a single gene allows early prediction of other (extra-pancreatic) clinical features associated with that specific gene, facilitating provision of necessary support and interventions soon after diagnosis; in the case of neonatal diabetes this would be in the first six months of life. This contrasts with previous approaches where clinicians would have to wait for the patient to develop extra-pancreatic features before determining which genes to sequence.[52] In neonatal diabetes a genetic diagnosis can now be made in 80% of cases,[52] because all babies who present with diabetes in the first 6 months of life can have a panel of known

disease-causing genes sequenced rapidly and accurately using the NGS method.

Humans with KCNJ11 mutations represent the best example of precision medicine in diabetes

A good example of precision medicine in monogenic diabetes is the treatment of *KCNJ11* neonatal diabetes with sulphonylureas.[53] *KCNJ11* encodes the Kir6.2 subunit of the pancreatic ATP-dependent potassium (K_{ATP}) channel; it is present in β cells and links blood glucose to insulin secretion. In 2004, the sequencing of *KCNJ11* in human subjects established mutations in this gene as a cause of permanent neonatal diabetes (PNDM).[54] PNDM affects ≈1/100,000 live births[55] and is defined as diabetes diagnosed within the first 6 months of life. To date there have been 24 genetic causes of neonatal diabetes identified,[52,56,57] and *KCNJ11* mutations are the commonest cause accounting for around one third of all cases.[52]

KCNJ11 mutations result in diabetes by rendering the K_{ATP} channel unresponsive to metabolically-generated ATP. Affected babies are clinically very sick and show insulin deficiency, with almost 80% presenting in diabetic ketoacidosis (DKA).[58] Until pathogenic variants in the *KCNJ11* gene were discovered these children were thought to have T1D and were treated with insulin injections.[54] Physiological experiments in affected individuals highlighted the possibility that sulphonylureas, used in T2D to bind and close the K_{ATP} channel, could be used as a targeted treatment option in *KCNJ11* PNDM. This was confirmed in 2006 when the first large cohort study showed that 90% of patients were able to switch from insulin injections onto oral

sulphonylureas with improvements in glycaemic control and less glycaemic variability.[53,59] Inability to switch, although uncommon, is associated with specific genotypes and long duration of diabetes before attempting to change treatment.[60,61] In those who switch successfully, the excellent initial glycaemic response is maintained over at least 5 years and is not associated with any increase in hypoglycaemia rates.[62-64]

The repurposing of an existing oral diabetes therapy that resulted in near normalisation of blood glucose for the great majority of affected individuals with *KCNJ11* PNDM was life-changing for patients and their families, and human research was crucial for this discovery. Indeed, without the gene discovery and the clinical trial of targeted therapy in humans, people with *KCNJ11* PNDM would have remained on a treatment that was not very efficient and that allowed only suboptimal glycaemic control, leading to increased risk of long-term diabetes complications.

Neurological features in KCNJ11 PNDM reflect expression of the KCNJ11 gene in the brain and vary according to genotype

Initial reports of *KCNJ11* PNDM showed that ≈20% of affected individuals exhibited overt and severe neurological features in addition to their diabetes; this was named DEND syndrome (developmental delay, epilepsy and neonatal diabetes - or intermediate DEND (iDEND) if epilepsy was not evident in the first 12 months of life. The clinical phenotype was found to be related to the genotype, with more severe clinical features being associated with the more functionally severe mutations.[49,66] For example, early studies reported developmental delay / intellectual disability (often severe), motor problems and /

or epilepsy in ≥80% of patients with the V59M mutation, in contrast to the R201H mutation where diabetes without neurological features was reported in >95% cases.[54,59,66-77]

The presence of neurological features in this type of diabetes is due to expression of *KCNJ11* in K_{ATP} channels in several brain regions as well as the pancreas, with particularly high levels of expression in the cerebellum.[78] Recent research has shown that in addition to the classical DEND syndrome, patients can have a range of other specific features. Neurodevelopmental disorders such as autism and ADHD are associated with the more functionally severe mutations like V59M.[79,80] Furthermore, a range of specific neuropsychological impairments affecting executive function, attention, praxis, working memory, vocabulary, and visuomotor performance have been identified.[79,81-83] Interestingly, subtle abnormalities are also observed in patients with mutations previously thought to cause diabetes alone e.g. R201H. One large cohort study of patients without overt neurological features reported attention deficits in all patients and dyspraxia (developmental coordination disorder) in 80%.[81]

Performing this detailed phenotyping in humans has provided clinical insights that would not have been possible using non-human research methods. For example, selective expression of the V59M mutation in the rodent brain gives rise to a model of DEND syndrome which shares characteristics with the human neurological phenotype.[46] However, there are also notable differences e.g. the mice show reduced anxiety behaviour whereas humans show more anxiety.[47,79] In addition, the milder neurological phenotypes associated with other mutations in the same gene have not been explored in rodent models,

and subtle cognitive deficits would be very difficult to assess in animals in the same way as they can be assessed in humans.

Impact of sulphonylureas on the neurological phenotype in KCNJ11 PNDM, and generating mechanistic hypotheses from the rodent model

In addition to achieving excellent metabolic control, an exciting aspect of switching patients with *KCNJ11* mutations from insulin to sulphonylureas, which was initially described in clinical case reports and neuroimaging studies, is an improvement in the neurological features.[76,84,85,86,87] This was recently confirmed by a prospective study which showed partial improvement in some of the neurological features in the first year after switching to sulphonylureas.[88] It has been suggested that the neurological response may be better the earlier in life the sulphonylureas are started,[83] due to increased neuroplasticity in younger children, but further studies are needed to address this issue.

Another possible reason for the incomplete CNS response to sulphonylurea treatment in people with *KCNJ11* PNDM is that therapeutic concentrations of sulphonylurea are not achieved in the human CSF. In rats, active transport of glibenclamide out of the brain across the blood brain barrier (BBB) has been demonstrated.[89] Therefore high concentrations of glibenclamide, as seen in the blood, are not achieved in the brain. This concept has led to clinical recommendations of higher doses of sulphonylureas in individuals with neurological features, with improvements reported by patients at doses of around 1mg/kg/day glibenclamide. These higher doses appear to be safe with no increase in rates of hypoglycaemia.[90] However, given the issues around translation of animal models outlined above, and the structural differences

between the rodent and human brain,[91] it will be important to confirm in future human studies how glibenclamide and other sulphonylureas are handled in the human CNS. This may include direct *in vivo* measurement of sulphonylurea concentrations in human cerebrospinal fluid (CSF), or the use of *in vitro* experiments with BBB models[92] which may provide a potential means of investigating this question without the risks of invasive procedures in patients.

Lessons learned from KCNJ11 PNDM are not directly applicable to all neonatal diabetes or to polygenic forms of diabetes

KCNJ11 PNDM is a good example of how human molecular genetics has driven the application of precision medicine in diabetes. However, *KCNJ11* mutations are only one cause of neonatal diabetes, and findings in one subtype are not generalisable to all, although the general concept of using molecular genetics to determine aetiology and treatment can be applied more widely (Figure 1). Other subtypes of neonatal diabetes are caused by mutations in a variety of genes; all share the clinical characteristic of diabetes in the first 6 months of life, but there are significant phenotypic differences between them. For example, people with neonatal diabetes due to insulin (*INS*) gene mutations (which account for around 10% of cases of neonatal diabetes) do not have any specific neurological phenotype[93], whereas CNS features comprise a large part of the phenotype in *KCNJ11* PNDM, as discussed above. Individuals with other syndromic forms of neonatal diabetes have neurocognitive impairments in addition to other multi-system features e.g. *GATA6* mutations cause cardiac defects, pancreatic exocrine insufficiency, gut abnormalities and hypothyroidism / hypopituitarism.[94]

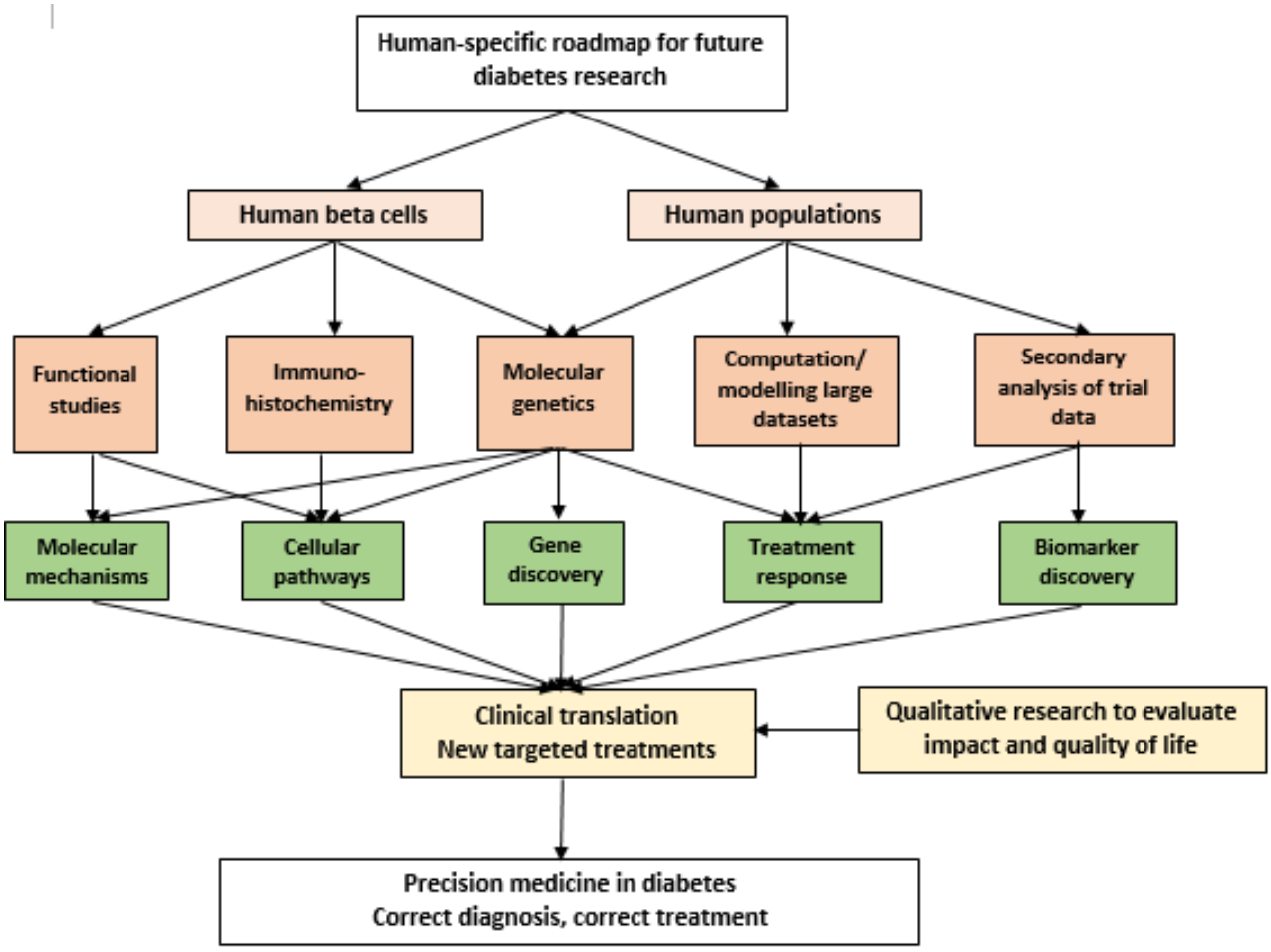

Figure 1; Human-specific research methods (orange boxes) can be applied to key areas (green boxes) relevant to diabetes pathophysiology, leading to development of new targeted treatments.

In addition to phenotypic differences, differing genetic aetiologies also means that different treatment approaches are needed. Heterozygous dominant negative *INS* mutations result in production of structurally abnormal preproinsulin and proinsulin within the beta cell, ER stress, cell death and absolute insulin deficiency.[52] This requires lifelong treatment with replacement doses of insulin[95], in stark contrast to the sulphonylurea-sensitivity of patients with *KCNJ11* mutations.[53] Even within *KCNJ11* neonatal diabetes, there is heterogeneity amongst patients with the same

mutation in terms of phenotype and treatment response, as described above. This heterogeneity is true for all subtypes of diabetes, including the common polygenic forms (T1D and T2D); however, it provides an opportunity to define discrete subgroups in a precise manner, with significant implications for new drug discovery and repurposing of existing treatments.

A human-specific roadmap for future diabetes research

We have established that findings obtained with animal models are not efficiently translated into humans, and it is impossible to generalise research findings from one subtype of human diabetes to another. Therefore alternative approaches are needed to drive advances in diabetes research that are clinically translatable. A range of rapidly evolving methods can be applied to human cells and human populations to enhance understanding in key areas, facilitating development of new targeted treatments between and within all subtypes of diabetes and allowing application of precision medicine (Figure 1).

The impact of molecular genetics in T1D and T2D – aetiology and correct diagnosis

Before we can develop effective new therapies in diabetes we must identify and understand aetiological pathways that can provide targets for treatment. One of the ways in which human molecular genetics has enhanced our understanding of the pathophysiology of polygenic diabetes is through genome wide association (GWA) studies.[96,97] These have been made possible by development of high throughput genotyping technologies such as NGS, increased availability of large cohorts of individuals with the disease in question

and control population data with which to compare them (see below), and better understanding of sequence pattern variation.[97] Over 100 T2D susceptibility loci have been identified to date, and there is now much focus on determining the function of associated genes and the pathways in which they play a role.[98] However, interpretation of the function of associated genetic variants is challenging as it is frequently difficult to prove a causal link between the variant and the disease.[98] In addition, effect sizes of causal variants in T2D are small,[97] making it extremely difficult to develop specific therapies targeted at a single gene or pathway, as has been described above for monogenic diabetes. For these reasons clinical translation of GWAS findings has been limited to date. In the future, as whole genome sequencing becomes less costly it is likely that larger populations will be screened which may assist the discovery of new variants or help explain existing associations and how they relate to T2D risk. Further, advances in functional experimental techniques may enhance our ability to move from associations to causal relationships. T2D GWAS will therefore be an important tool in terms of biological insights, drug targets, and disease prediction (Figure 1).

Despite the complexities of functional interpretation of genetic risk variants in polygenic diabetes, they can be useful in assisting diagnosis, which is fundamental for selecting the correct treatment. In T1D, a genetic risk score (T1D GRS) comprising 30 T1D-associated risk variants each weighted according to individual risk contribution, has been developed; this score can reliably differentiate T1D from T2D, and T1D from monogenic diabetes.[99,100] The T1D GRS is now being used in both research and clinical contexts. This has significant implications in terms of making the correct clinical diagnosis early and starting the correct treatment, as well as ensuring phenotypic purity in

research cohorts in T1D. As it is a relatively low-cost investigation, its use is likely to become more widespread in the future and there is potential for similar methodology to be applied to other polygenic diseases.

Finally, genes involved in monogenic diabetes may also be implicated in polygenic disease, for example activating mutations in *KCNJ11* cause neonatal diabetes whilst the common E23K variant in *KCNJ11* has been associated with T2D susceptibility.[54,101] Therefore monogenic diabetes has utility in identifying potential mechanisms that contribute to polygenic diabetes risk.[102] However, the complex inheritance patterns, multifactorial aetiologies and small effect sizes of genetic risk variants in polygenic diabetes give rise to very heterogeneous populations of patients, and multiple complementary approaches are required to unpick this.

Availability of large population based data sets and sharing of data can provide new insights into polygenic diabetes

Historically, one of the drawbacks of research in humans has been the inability to power studies adequately due to lack of availability of cohorts of patients with a specific disease or aspects of a disease of interest. This is particularly problematic in genetics studies, where large populations are required to identify risk variants with relatively small effect sizes in polygenic diseases like T1D and T2D. In recent years, the problem has been mitigated by the availability of increasing numbers of large-population research cohorts, such as UK Biobank.[103] UK Biobank contains anonymized health data, including genetic and clinical information, on over 500,000 volunteers which is available for approved researchers to use. Application of rapidly advancing bioinformatics

techniques to these population-based datasets represents an exciting opportunity to gain novel insights. Indeed, one recent publication using UK Biobank and applying the T1D GRS outlined above[100] provided new insights into T1D, by demonstrating persistence of T1D risk beyond the age of 30 thereby highlighting the need for clinicians to continue to consider this diagnosis in adults.[104]

Another means of acquiring data from large populations is data sharing from large-scale clinical trials, which is now actively encouraged and endorsed by many trial sponsors and influential bodies.[105] Full individual participant data for many trials can be requested and accessed by researchers via websites such as Clinical Study Data Request.[106] Secondary analysis and statistical modelling of trial data allows evaluation of outcomes in subgroups of patients based on clinical characteristics, presence of specific biomarkers, or genotype. These population-based methods can facilitate an alternative approach to precision medicine in polygenic forms of diabetes, such as T2D, whereby clinical features and biomarkers are used to stratify patients into specific treatment groups.[13] An excellent example of this is using clinical features to stratify patients with Type 2 diabetes when deciding which second-line glucose lowering therapy to use.[107] Therefore, future clinical research in diabetes will rely heavily on shared human population data.

The concept of large scale data sharing is also applicable to genetic data. NGS technologies have reduced the cost of genetic testing by a factor of between 100 and 200 in the last 5 years. As genetic testing continues to decrease in price and analysis methods improve, sequencing particularly at the level of exome or whole genome will become more accessible to larger numbers of individuals. This will generate vast quantities of genetic data requiring accurate

interpretation, which can be a major challenge. However, in recent years databases generated from data sharing containing genetic variants from human populations (e.g. the Genome Aggregation Database (gnomAD), Exome Aggregation Consortium (ExAC), dbSNP) and human disease (e.g. the Human Gene Mutation Database (HGMD), ClinVar) have revolutionised the ability of clinical scientists to interpret variants and their likely pathogenicity. Further, initiatives such as the 100,000 genomes project seek to not only provide clinical diagnoses for people with rare genetic conditions, but also to generate a large population sample of genomic data that will be invaluable for researchers in the future as the patients' health records and outcome data can be linked to their genetic data.[108]

Indeed, the concept of integrating research with clinical practice has evolved substantially in recent years, particularly where there is availability of Electronic Health Records (EHR). Primary Care is particularly well placed to apply this because many practices have moved to an EHR approach. In the UK, Clinical Practice Research Datalink (CPRD) is a well-established source of anonymised clinical information from General Practice (GP) records that can be utilised for research; it has resulted in over 1800 publications to date.[109] Most patients with diabetes are followed up clinically by their GP therefore this is a key opportunity for research in the field. Indeed, it has been shown that diabetes and its treatment are two of the main topics of research being generated from primary care databases in the UK.[110] However, there are several legal and ethical issues relating to data sharing and storage that have hindered the use of EHR in many healthcare settings; this is particularly pertinent when it comes to linking genomic data with personally identifiable data.[111,112] To make the most of the opportunities afforded by EHR in the future, robust policies

addressing confidentiality and security of information should be developed by key regulatory authorities.[113]

A caveat of the clinical and genomic data repositories that are currently available is the paucity of ethnic diversity in the populations studied leading to under-representation of non-European groups.[114] The ever-increasing numbers of individuals contributing data to such repositories bodes well for improved stratification by ethnicity in the future, but in the meantime caution should be used when attempting to generalise findings to minority populations.

Availability of human islets for research and new experimental techniques provide insights into pathways involved in diabetes pathophysiology

Modern immunohistochemical and imaging techniques and availability of collections of specific human tissues for research can greatly enhance our understanding of the pathophysiology of diabetes. A recent study of pancreas sections obtained at post-mortem from a UK cohort of patients with T1D provided exciting mechanistic insights, demonstrating a different insulitic profile in patients diagnosed under 7 years versus those diagnosed over 13 years.[115] In addition, the latter group retained ~40% of insulin containing islets at diagnosis, which implies β cell dysfunction as opposed to loss may be important. This work and ongoing research in the field will have important implications for patient stratification in T1D immunotherapy trials and in the development of targeted treatments for specific patient groups.

Research in human islets harvested from cadaveric donors has also advanced knowledge relating to cellular and molecular pathways relevant to T2D. Recent advances in genetic techniques have facilitated identification of many T2D

susceptibility genes and allowed genetic data to be combined with functional data to map pathways and define mechanisms associated with human islet dysfunction, including key regulatory networks.[116,117] These approaches have great potential to further enhance our understanding of polygenic forms of diabetes and gene-environment interactions, and in combination with findings from large population studies, to guide development of new therapeutic interventions.

Precision medicine must also encompass patient preference and impact on quality of life

Another area of precision medicine where human studies are essential is exploring the influence of psychosocial factors on patient outcomes. Quality of life measures are frequently used in evaluating cost-effectiveness of medical interventions.[118] Development of targeted treatments for specific subtypes of diabetes should therefore include research that evaluates patients' perceptions of these treatments and impact on quality of life. Even when the biological efficacy of new treatments has been proven, the willingness of patients to accept them will be variable and influenced by psychological factors. For example, treatment change from insulin injections to oral sulphonylureas had a hugely positive impact on many families affected by *KCNJ11* neonatal diabetes. They experienced improved quality of life, more freedom and reduced levels of psychological distress as a result of better glycaemic control, less glycaemic variability and reduced need for hypervigilance of parents towards their affected children.[119-121] However, for a few adults with *KCNJ11* mutations who had been assumed to have Type 1 diabetes all of their lives, there was initial uncertainty about the implications of a genetic diagnosis as it could result in a

loss of the insulin injections on which they had always been dependent.[119,120] These individuals viewed insulin very much as part of their identity and loss of this identity required significant adjustment.[122]

In addition, mental illness is a significant problem in individuals with chronic physical health conditions. The incidence and prevalence of depression is increased in people with diabetes[123], which will have implications for adherence, response and attitudes to new treatments. Severe mental illness such as schizophrenia and bipolar disorder are associated with a 2-3 fold increase in diabetes prevalence and this is only partly explained by the adverse metabolic effects of antipsychotic treatment.[124] Patient stratification using only biomarkers or genetic risk variants for diabetes does not take account of psychological influences and psychiatric co-morbidity. Future models for precision approaches in diabetes should incorporate these ideas; this will be challenging but could be facilitated by integration of qualitative methods into biological studies and inter-disciplinary collaboration.

Human-specific research can enhance understanding of heterogeneity and is the first step towards precision medicine across all subtypes of diabetes

In diabetes, the correct diagnosis is essential to ensure the correct treatment is given. However, both diagnostics and therapeutics continue to represent significant challenges to diabetologists. Heterogeneity between and within subtypes of diabetes is becoming increasingly recognised and only serves to make the task more difficult. To enable a precision medicine approach in diabetes, we need to significantly enhance our understanding of this heterogeneity.

Animals have been used historically to model diabetes in humans, but their utility is limited especially as the emphasis in humans is on specific treatments for specific diabetes subtypes. The animal models used have fundamental genetic and phenotypic differences to diabetes in humans and cannot reflect the diversity of subtypes. This is exemplified by the lack of effective translation of treatments developed in animal models into humans. Therapeutic advances in diabetes therefore require alternative human-specific research methods.

Monogenic diabetes is an excellent example of the application of precision medicine. In particular, the treatment of *KCNJ11* neonatal diabetes with sulphonylureas represents the best precision approach in diabetes and illustrates how advances in human molecular genetic techniques have facilitated major discoveries, with huge implications for patient care. However, it also illustrates how specific targeted treatment for one subtype within a broader category (in this case neonatal diabetes) cannot be generalised to all subtypes. In polygenic diabetes such as T1D and T2D, genetics can help by providing information about risk variants but effect sizes are small. The situation is particularly complex given that within T1D and T2D there is significant heterogeneity between groups of individuals, whether they are defined by clinical characteristics or response to treatment.

In summary, the road ahead in diabetes research is exciting but complex. A combined approach that uses advanced molecular genetic techniques, pathway-focused research in human islets, computational methods in large population cohorts and trial data, qualitative research, and other techniques yet to be developed, may help to unpick the differences between diabetes subtypes. This will be the first step towards understanding and rising to the

challenge of heterogeneity in diabetes, to facilitate precision medicine and improved clinical care.

Conflicts of Interest

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METHODS

This section describes the methods used in the data chapters, focusing on those aspects not already covered in detail in the publications relating to each study. Copies of the relevant documents are included in Appendix 1.

CHAPTER 1

In our long-term follow-up of patients with *KCNJ11* PNDM, data collection forms were sent to participating clinicians to complete using the clinical records of their patients. These forms contained fields relating to glycaemic control (including HbA1c and any episodes of hypoglycaemia or ketoacidosis), side effects, diabetes complications, puberty and development, other cardiovascular risk factors, and neurological features and whether these improved following transfer from insulin to sulphonylureas. A copy of the data collection form can be found in Appendix 1.

CHAPTER 2

Meal plans and preparation

In our study of the physiological response to different foods in people with sulphonylurea-treated *KCNJ11* PNDM, meals were designed with the assistance of a diabetes dietician taking into consideration palatability and quantity required to achieve the desired proportions of given nutrients. We opted for solid meals using everyday foods as opposed to a liquid meal to enhance generalisability to the 'real-life' context. The content of the carbohydrate and protein meals are shown in Tables 1, 2a and 2b in Appendix 1. Three months into the study the original brand of ham used was discontinued therefore subsequent protein meals used a different brand of ham,

the quantity of which was adjusted to achieve near-identical proportions of nutrients to the original protein meal.

Fasting guidelines

Prior to each visit participants were asked to follow fasting guidelines as follows;

- Eat normally on the day prior to the test.
- Do not consume any alcohol for 48 hours before your visit.
- Do not have anything to eat or drink (except water) from 10pm on the night prior the blood test. Please drink plenty of water prior to your appointment but do not add anything to it. You must not drink tea or coffee.
- Do not consume any paracetamol or paracetamol-containing medicines for 24 hours before your visit. Please take all other medications as normal apart from those that are to be taken with food (these are to be taken later with breakfast).
- Exercise will affect your blood sugar so do not exercise excessively for 48 hours prior to your visit.
- Do not smoke on the morning of the test.

Baseline data collection forms

Baseline data comprising height, weight, medications and time of last food / drink were collected at each visit using the data collection form in Appendix 1.

Hypoglycaemia questionnaires

To screen for subjective symptoms of hypoglycaemia throughout the study visits, participants were given a questionnaire at each blood sampling time point. This used a Likert scale (1-7, where 1 is absent and 7 is severe) to score

neuroglycopenic and autonomic symptoms as previously described (1, 2) (Appendix 1).

Blood sampling, handling and storage

Blood samples were obtained, processed and stored as per the sample handling / processing standard operating procedure (SOP) and study flowchart in Appendix 1. These were developed in accordance with standard procedures employed by the Exeter NIHR Clinical Research Facility (CRF) and the Royal Devon and Exeter Hospital biochemistry laboratory. Biochemical analysis of samples is described in the methods section in chapter 2.

CHAPTER 3

Medical and developmental history

A medical and developmental history was obtained from the parent(s) of participating children. This included information about diabetes presentation, sulphonylurea transfer and glycaemic control, any other medical problems (including epilepsy, previous psychiatric diagnoses and neurological features), current medications, family history, pregnancy and birth, ages at which major milestones (gross motor, fine motor, social / emotional and speech / language) were attained, education and learning support required.

Psychiatric Evaluation

The Development and Wellbeing Assessment (DAWBA) (3) and Strengths and Difficulties Questionnaire (SDQ) (4) are described in detail in chapter 3A. Appendix 1 contains copies of these questionnaires for reference.

Neuropsychological Tests

A selection of neuropsychological tests from different standardised batteries was used, with advice and guidance from the Consultant neuropsychologist in the study team. The objective was to assess a broad range of cognitive domains whilst ensuring that the specific areas of difficulty that had been highlighted by affected individuals and families or described in previous studies were included (5). Neuropsychological testing was undertaken at a neonatal diabetes family day event, by medical professionals in the research team who had been trained in administering the tests by the study Consultant neuropsychologist. Tests were scored by the Consultant neuropsychologist and neuropsychology assistant. Below is a description of the tests used and the standardised batteries to which they belong;

Developmental Neuropsychological Assessment (6) explores the 6 domains of attention and executive functions. For the purpose of this study we used only the 'narrative memory' subscale, which measures episodic memory, as the child is required to listen, store and recall a story. Children are asked to freely recall the story and are then cued with age-appropriate questions relating to it.

Delis-Kaplan Executive Function System (7) tests various aspects of executive functioning. We used the 'verbal fluency' subtest, which involves the young person being asked to generate as many words as possible in 1 minute using the letter 'F', and then to repeat this for words beginning with 'A', then 'S'. No names of people or places should be used. The task is a test of abstract thought and cognitive flexibility whilst retaining rules.

Wechsler Intelligence Scale for Children (8) measures full scale IQ. We used three subscales of the WISC-IV. The first was the 'symbol search' task

measuring processing speed; it is a timed pen and paper test that assesses the ability to focus attention and quickly scan, discriminate between, and order visual information. The young person is asked to identify if a symbol presented on the left of the page is in the block of symbols on the right of the page. The second subtest, 'digit span', assesses memory capacity and working memory, by measuring how many numbers can be remembered (forwards and in reverse) after they are read to the child by the examiner. The 'vocabulary' subtest measures verbal comprehension. Words of increasing difficulty are read to the child and they are asked to define the words. The test is scored based on sophistication of the definition given. Reliability coefficients for the subtests used are good (symbol search (.79), digit span (.87) and vocabulary (.89)) (9).

Beery-Buktenica Test of Visual Motor Integration (10) involves written tasks used to assess fine motor skills, visual perception and visual-motor integration ('hand eye coordination'). The visual perception test presents the child with a series of geometric shapes of increasing complexity and underneath a series of shapes which are similar. The child's task is to identify the shape that is exactly the same. The motor coordination test requires children to copy increasingly complex geometric shapes within predetermined lines. The visual motor integration test requires the children to copy predetermined increasingly complex geometric shapes but with no guidance (10).

CHAPTER 4

All participants were assessed at home by the Consultant neurologist and Consultant neuropsychologist in the research team. Each participant underwent a comprehensive neurobehavioural assessment.

Medical and developmental history and neurological examination

The Consultant neurologist obtained a medical history, with an informant present if possible. This included information about the participants' early presentation with diabetes, episodes of hypoglycaemia, seizures, any other medical problems, medications, family history and social and occupational history. A developmental history was also taken, which included information about the pregnancy and birth, ages at which major milestones (gross motor, fine motor, social / emotional and speech / language) were attained, education and any learning support required. A full neurological examination was performed which involved assessment of tone, power, reflexes, coordination, and sensation, as well as a cranial nerve examination and some simple tests of motor sequencing and praxis.

Questionnaires administered to participants / informants

The Autism-Spectrum Quotient (AQ) (11, 12) (Appendix 1) contains 50 items relating to social skills, attention, communication and imagination. The participant selects one response for each statement (definitely agree, slightly agree, slightly disagree, definitely disagree). One point is given for each response that is associated with autism spectrum disorder (ASD), therefore higher scores indicate more autistic traits, with the clinical threshold being 32 points or higher (11).

The Hospital Anxiety and Depression Scale (HADS) questionnaire (13) (Appendix 1) is a mood assessment consisting of a series of 14 statements which the participant reads and rates from 0-3. There are different statements relating to depression and anxiety, which are scored separately; a score of 11

or greater is the threshold for clinically significant depression or anxiety symptoms.

The Cambridge Behavioural Inventory Revised questionnaire (14) (Appendix 1) is a functional and behavioural assessment which is completed by an informant / carer who knows the participant well. It is described in detail in chapter 4.

Neuropsychological tests

The Consultant neuropsychologist administered and scored a series of pre-selected neuropsychological tests from standardised batteries. As per the paediatric study, tests were selected to capture key areas of difficulty highlighted previously by patients and in the literature (5, 15, 16) whilst obtaining information on a broad range of cognitive domains. The neuropsychological tests used are described in Chapter 4 and summarised briefly below;

- The verbal paired associates and visual reproduction subtests of the Wechsler Memory Scale (WMS-IV) (17, 18) were used to assess verbal and non-verbal (visual) memory in terms of recall and recognition. Verbal paired associates involves the examiner reading pairs of words to the participant and then presenting them with a word from each pair and asking them to recall the other word. Visual reproduction involves the participant looking at designs for 10 seconds each and then drawing the designs from memory.

- The Wechsler Abbreviated Scale of Intelligence (WASI) was used as a brief measure of current IQ (19). The vocabulary subtest involves the participant providing definitions for words and images provided by the examiner. The matrix reasoning subtest provides a series of patterns to the participant in

grids that are not fully complete; the participant has to choose the response that best fits each pattern.

- The cancellation and digit span subtests of the WAIS-IV (18) were used to assess processing speed and working memory. The cancellation subtest involves the participant looking at a sequence of pictures and then crossing out specific target pictures. The digit span subtest is administered as described above for the paediatric study.

- The Colour Trails Test CTT1 and CTT2 were used to assess attention and hand-eye coordination (20). The CTT1 is a timed pen and paper test that involves the participant connecting circles numbered 1-25 in sequence. The CTT2 applies the same principle but the participant alternates between different colours (21).

- The Controlled Oral Word Association Test (COWAT) (22) was used as a test of executive function. It is administered as described above for the 'verbal fluency' subtest in the paediatric study.

- The Visual Object and Space Perception battery (VOSP)(23) was used to assess visuospatial function. The incomplete letters and object decision subtests measured object perception. Incomplete letter involves a series of letters with parts missing being presented to the participant, who is asked to identify them. Object decision involves different stimuli being shown to the participant who has to select the real shape from the distractor stimuli. The dot counting and cube analysis subtests assess spatial perception. In the former, the participant counts black dots against a white background. In cube analysis, the participant has to identify the number of cubes on boards showing a series of solid structures.

- The Addenbrooke's Cognitive Examination-Revised (ACER) (24) (Appendix 1) was used as a broad cognitive screen, assessing attention/orientation, memory, fluency, language, and visuospatial function.

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CHAPTER 1

Effectiveness and safety of long-term treatment with sulphonylureas in neonatal diabetes due to *KCNJ11* mutations: an international cohort study

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ATH was involved in study design, protocol development, data collection, data analysis, data interpretation, and writing the report. ÅS, and PRN were involved in study design, protocol development, data collection, data analysis, data interpretation, sample collection and analysis for the physiology studies and writing the report. FB, JB, MP, and DI were involved in study design, protocol development, data collection, data interpretation and critically revising the report. PS, PBJ, TS, were involved in sample collection and analysis for the physiology studies and critically revising the report. EC, EHT, ERP, SEF, TB, NJT, MHS, SE, IK, and MS were involved in data collection, data interpretation and critically revising the report. All authors reviewed and approved the manuscript for submission.

MY CONTRIBUTIONS TO THE CHAPTER

I designed the study and developed the protocol with ATH, PRN, FB, JB, MP, ÅS and DI. I collected data on all study participants with support from all co-authors and Ms Frances Mathews in her role as research assistant. I performed all analyses of the data except those associated with the physiology studies, which were done in Norway. I interpreted the data with support from ATH, PRN, ÅS, FB, JB, MP, and DI. I produced all figures and tables except those relating to the physiology studies. I wrote the manuscript, which I revised according to the feedback I received from all co-authors.

RESEARCH IN CONTEXT

EVIDENCE BEFORE THIS STUDY

A large cohort study established in 2006 that high dose sulphonylureas could be used to treat permanent neonatal diabetes (PNDM) due to *KCNJ11* mutations. This was life-changing for patients as it allowed 90% to stop insulin injections and achieve better glycaemic control in the short-term (1 year) without any increase in hypoglycaemia. The short-term benefit of transferring to sulphonylureas has been replicated in many follow-up studies since then. A key question is whether this excellent outcome is maintained in the long-term, particularly as in type 2 diabetes after 5 years on therapy ~44% patients show sulphonylurea failure requiring additional therapies to maintain glycaemic control. Furthermore, in type 2 diabetes sulphonylureas have been associated with hypoglycaemia, raising a safety question especially as high doses (typically 0.2-0.8mg/kg/day glibenclamide) are used in PNDM compared to the lower doses used in type 2 diabetes (typically ~0·1mg/kg/day glibenclamide).

We searched PubMed with the terms “*KCNJ11*”, “kir6.2”, “neonatal”, “diabetes", “sulphonylurea”, "sulfonylurea”, “glibenclamide”, “glyburide”, “therapy”, “treatment”, to identify follow up studies. Only a few small (n<11) relatively short term (2.5-5.7 years) series have been reported with the best study to date reporting maintenance of good control in 11 patients from a single centre followed up for a median of 5.7 years. Prior to our study it was not known when PNDM due to *KCNJ11* mutations was treated with sulphonylurea therapy in the long-term (10 years) if the glycaemic control would be maintained, whether this

long-term therapy was safe and what would be the long-term impact on neurological features.

ADDED VALUE OF THIS STUDY

To our knowledge, this is the first study of the long-term efficacy and safety of sulphonylureas in a large multi-centre international cohort with *KCNJ11* PNDM. We show that sulphonylurea failure, seen commonly in type 2 diabetes, is not a feature of *KCNJ11* PNDM. Sulphonylureas are safe long-term even in high doses in this unique group of patients and excellent glycaemic control is maintained over 10 years. Despite initial improvement in some patients, neurological features persisted on long term sulphonylureas.

IMPLICATIONS OF ALL THE AVAILABLE EVIDENCE

All babies diagnosed with diabetes under 6 months of age should undergo rapid genetic testing to facilitate early transfer of those with *KCNJ11* mutations to sulphonylureas as first line treatment, and this should be expected to result in safe long lasting excellent glycaemic control for at least 10 years but neurological features are likely to persist..

ABSTRACT

BACKGROUND

KCNJ11 mutations cause permanent neonatal diabetes (PNDM) due to pancreatic KATP channel activation. 90% of patients successfully transfer from

insulin to oral sulphonylureas with excellent glycaemic control initially. It is not known if this outstanding example of precision medicine is maintained in the long term. Sulphonylurea failure is seen in ~44% of people with type 2 diabetes after 5 years of treatment. We report the first 10-year follow-up of sulphonylurea efficacy and safety in a large international cohort of patients with *KCNJ11* PNDM.

METHODS

We followed up 81 patients who transferred from insulin to sulphonylureas before December 2006. Primary outcomes were sulphonylurea failure and metabolic control. Secondary outcomes were adverse effects of sulphonylureas, diabetes complications, insulin secretory response, and impact of sulphonylurea therapy on neurological features. This study is registered with ClinicalTrials.gov, number NCT02624817.

FINDINGS

Median follow-up duration was 10.2 years. At recent follow-up, 75/81 (93%) remained on sulphonylureas alone. Excellent glycaemic control was maintained; median HbA1c pre-transfer to sulphonylureas was 8.1% (65.0mmol/mol), falling to 5.9% (41.0mmol/mol) at 1 year, $p<0.0001$ and 6.4% (46.4mmol/mol) at 10 years, $p<0.0001$. Median sulphonylurea dose decreased (1-year 0.30 mg/kg/day, 10-year 0.23 mg/kg/day, $p=0.03$). There were no reports of severe hypoglycaemia in 809 patient years on sulphonylureas. Eleven patients (14%) reported mild, transient side-effects but did not need to stop sulphonylureas. Seven patients (9%) had microvascular complications: they were on insulin longer compared to those without complications (median age at transfer 20.5

vs. 4.1years, p=0.0005). Despite initial improvement in some patients, neurological features persisted despite long-term therapy with sulphonylureas.

INTERPRETATION

Patients with *KCNJ11* PNDM should be treated with high dose sulphonylurea therapy from diagnosis as this therapy is safe and highly effective, maintaining excellent glycaemic control for at least 10 years.

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INTRODUCTION

Background

The best example of precision medicine in diabetes is the treatment of neonatal diabetes with sulphonylurea therapy [1]. Mutations in *KCNJ11* resulting in activation of the pancreatic K_{ATP} channel are the commonest cause of permanent neonatal diabetes (PNDM) [2,3]. A genetic diagnosis is crucial as at least 90% of patients can transfer from insulin injections to oral sulphonylureas, which bind to the SUR1 component of the K_{ATP} channel resulting in channel closure enabling insulin secretion [4-6]. Following transfer to sulphonylureas, patients have improved glycaemic control at 1 year without increase in hypoglycaemia [6] and less glycaemic variability [4,5].

A key question has been whether the excellent results in neonatal diabetes will be maintained or whether there will be sulphonylurea failure or adverse side effects with long-term therapy. Sulphonylurea failure, where sulphonylurea therapy no longer maintains good glycaemic control, is seen in ~44% of people with type 2 diabetes after 5 years of treatment [7]. The only follow-up studies have shown that the glycaemic response to sulphonylureas is maintained in *KCNJ11* PNDM but these have been single cases or small single centre cohorts (all n≤11) and of short duration (between 2.5-5.7 years) [8-10]. Furthermore, there are safety issues; hypoglycaemia is a known side effect of sulphonylurea treatment in type 2 diabetes, particularly in relation to glibenclamide [11] which is the sulphonylurea commonly used to treat *KCNJ11* PNDM. It is not known if hypoglycaemia and additional side effects will occur as a result of the long-term use of much higher doses of sulphonylureas in *KCNJ11* PNDM compared to type 2 diabetes (0·45mg/kg/day vs. ~0·1mg/kg/day glibenclamide) [6].

The long-term sustainability of therapy in *KCNJ11* PNDM is an important question as in many other areas of precision medicine the initial excellent results have not been maintained. For example, in oncology, long-term outcomes in clinical studies have been disappointing, primarily due to heterogeneity within tumours allowing selection and proliferation of subclones of cancer cells that are resistant to treatments targeted at specific pathways [12].

In addition to diabetes, patients with *KCNJ11* mutations exhibit central nervous system (CNS) features, owing to expression of *KCNJ11* in the brain as well as the pancreas [13]. CNS features range from the overt and severe DEND / iDEND syndrome comprising developmental delay, epilepsy and varying degrees of muscle weakness / hypotonia [14], to neurodevelopmental problems

such as autism and Attention Deficit Hyperactivity Disorder (ADHD) [15], to more subtle neuropsychological deficits, specifically inattention, dyspraxia and executive dysfunction [16,17]. A recent prospective study showed that sulphonylurea treatment results in a partial improvement in the CNS features in people with *KCNJ11* PNDM in the first year of therapy [18]. However, the initial CNS response is not as marked as the glycaemic response, which may be in part due to active transport of glibenclamide out of the brain, resulting in sub-therapeutic concentrations in the cerebrospinal fluid (CSF) [19]. An important question, which has not to our knowledge been investigated by any studies to date, is whether long-term therapy with sulphonylureas has an impact on CNS features in *KCNJ11* PNDM.

To address the key questions relating to long-term efficacy and safety of sulphonylureas in *KCNJ11* PNDM, we performed a 10-year multi-centre follow-up of a large (n=81) international cohort of patients with this rare form of diabetes.

METHODS

Study design, setting and patient selection

All patients diagnosed with *KCNJ11* PNDM in laboratories in Exeter, Rome, Bergen, Paris, and Poland, who transferred from insulin to oral sulphonylureas prior to December 2006, were eligible for the study. Clinicians collected clinical characteristics and annual data relating to glycaemic control, sulphonylurea dose, severe hypoglycaemia, side-effects, diabetes complications, and growth. Height and BMI were converted to SDS using WHO reference ranges [20]. Patients aged >19 years were assigned an age of 19 for calculating BMI SDS.

CNS features, both neurological and psychiatric, were documented prior to transfer and at most recent follow-up. Clinicians were specifically asked about clinical characteristics frequently associated with *KCNJ11* mutations (developmental delay, learning difficulties, epilepsy, muscle weakness, autism, ADHD, sleep problems, anxiety) [15,17,18,21,22] and whether there was an improvement in CNS features at the time of transfer to sulphonylureas. In cases where the sulphonylurea used was not glibenclamide, the dose was expressed as a percentage of the maximum recommended daily dose (as per British National Formulary) and converted to an equivalent dose of glibenclamide [23]. Hypoglycaemia was defined as severe if the patient had a seizure, loss of consciousness or was admitted to hospital for intravenous glucose or glucagon, as per International Society for Paediatric and Adolescent Diabetes criteria [24]. The research was conducted in accordance with the Declaration of Helsinki. Clinical data was collected during the course of the patients' routine care and was anonymised for use in the study. Informed consent was obtained from all patients or parents for participation in the physiological studies.

Physiological studies

We performed oral and intravenous glucose tolerance tests in 6 patients on sulphonylurea treatment for a median time of 9·8 years (6·7-11·4). (Supplementary Appendix).

Outcomes

The primary outcomes were sulphonylurea failure, defined as permanent re-introduction of daily insulin, and metabolic control, specifically HbA1c and

sulphonylurea dose. The secondary outcomes were severe hypoglycaemia, side-effects, diabetes complications, growth, and effects of sulphonylurea therapy on CNS features.

Statistical analysis

Data was analysed in Stata 14·0. Nonparametric statistical methods were used; Wilcoxon test for paired and Mann-Whitney test for unpaired data. Unless otherwise stated, results are presented as median (interquartile range [IQR]). A primary objective of the study was assessing the number of patients needing reintroduction of insulin and the time to reintroduction of insulin therefore a Kaplan-Meier survival analysis was used (see Supplementary Appendix, statistical methods for details).

For analysis of longitudinal annual follow-up data, we used values that were nearest to the transfer date anniversary. When values were not available within 6 months either side of the anniversary of transfer, missing values were imputed (for year 1 data we included values from 3 months to 1.5 years) (see Supplementary Appendix, statistical methods for details) [6]. Where possible HbA1c and sulphonylurea dose were recorded on the same date within each year. When this was not possible the HbA1c and sulphonylurea dose were measured as close together as possible within the same year. In 2 patients, who were on insulin due to pregnancy at most recent follow-up, data from the most recent pre-pregnancy review was used. Patients who had received a short course of insulin treatment at any point during the follow-up but had subsequently transferred back to sulphonylureas, and patients who required

small occasional (non-replacement) doses of insulin, were assigned to the sulphonylurea only group.

Data on hypoglycaemia was compared with a cohort from the Norwegian Childhood Diabetes Registry of 664 Norwegian patients with type 1 diabetes of duration mean(SD) 10.8(2.2) years, followed up for >8 years from diagnosis.

This study is registered with ClinicalTrials.gov, number NCT02624817.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

RESULTS

The numbers of individuals in each stage of the study are shown in Figure 1. Ninety patients were eligible for inclusion and 81 (90%), were enrolled in the study and provided long-term (>5·5 years) outcome data.

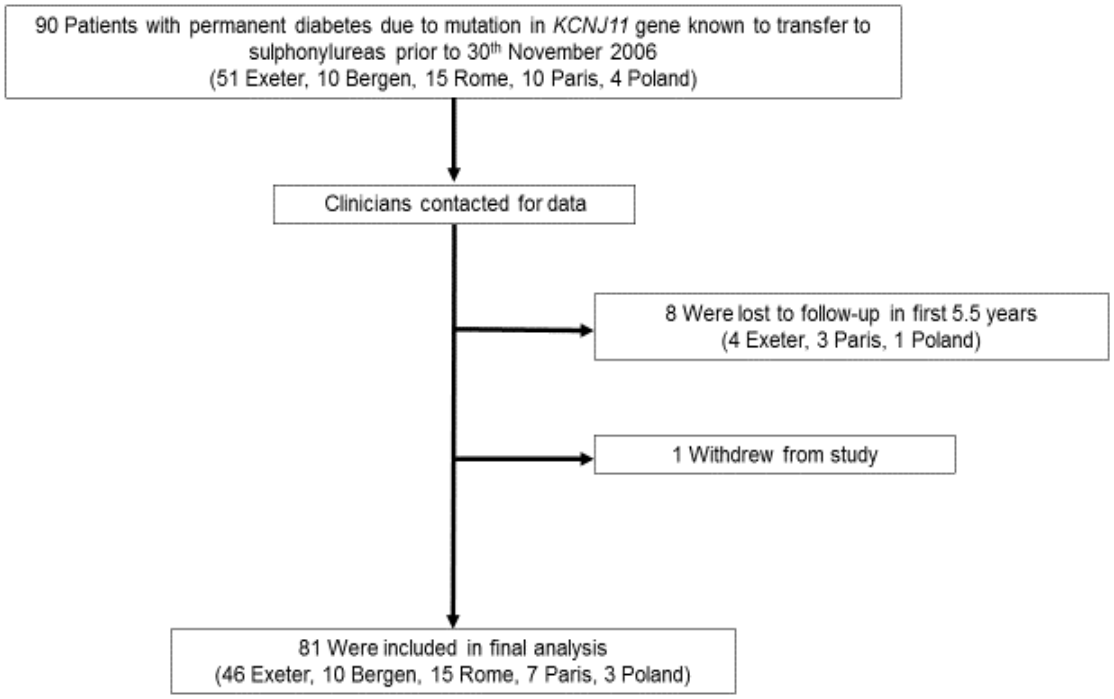

Figure 1. Overview of Study Design.

Flow diagram showing cohort selection and follow-up in accordance with Strengthening the Reporting of OBservational studies in Epidemiology (STROBE). Long-term follow-up was achieved for 90% of the eligible patients. Duration of follow-up was median(IQR) 10.2(9.3-10.8) years. We used 5.5 years (year 6 data) as cut-off for the term 'long-term' follow-up.

The clinical characteristics of patients are shown in Table S1 in the Supplementary Appendix. All patients were diagnosed with diabetes under 6 months and transferred from insulin to sulphonylureas between 0·2 and 34·5 years. The subjects not followed up were similar to the subjects in the study except they were older at transfer from insulin to sulphonylureas and younger at diabetes diagnosis (Table S1, Supplementary Appendix). Duration of follow-up for the cohort was median (IQR) 10·2 (9·3-10·8) years.

Sulphonylurea therapy remained highly effective, with 75 of 81 (92·6%) remaining on sulphonylureas without regular insulin at most recent follow-up (Figure 2A). No patient stopped sulphonylureas. Excellent glycaemic control was maintained and sulphonylurea dose fell over 10 years (Figure 2B). In patients remaining on sulphonylureas alone, HbA1c pre-transfer, at 1 year and most recent follow-up (median 10·3 years) was 8·1 (7·2-9·2)% (65.0 (55.2-77.1)mmol/mol), 5·9 (5·4-6·5)% (41.0 (35.5-47.5)mmol/mol) and 6·4 (5·9-7·3)% (46.4 (41.0-56.3)mmol/mol) (n=64). In the same patients, sulphonylurea dose (median[IQR]) at 1 year and most recent follow-up was 0·30 (0·14-0·53) vs. 0·23 (0·12-0·41) mg per kg per day (p=0·03, n=64).

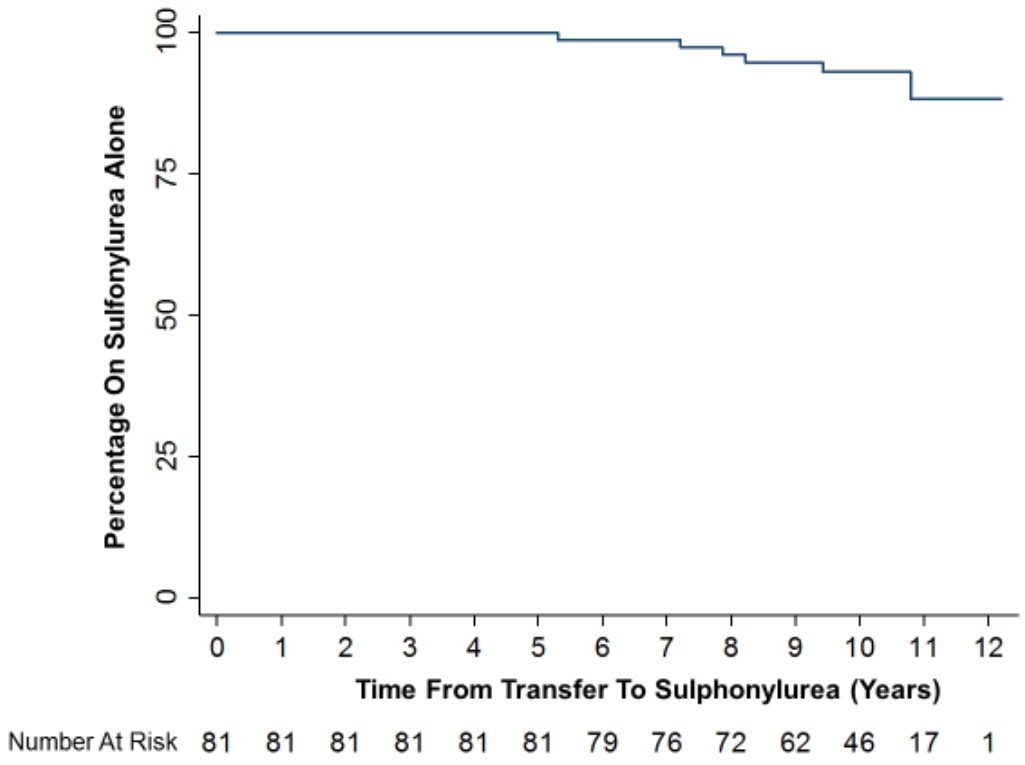

Figure 2A

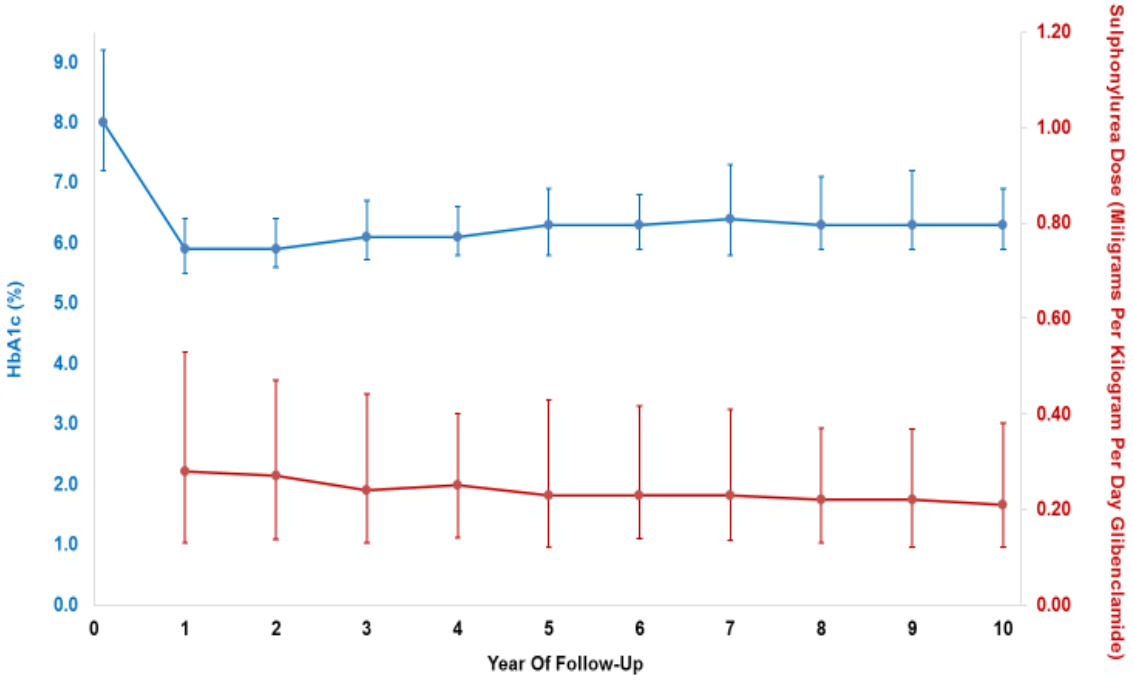

Figure 2B

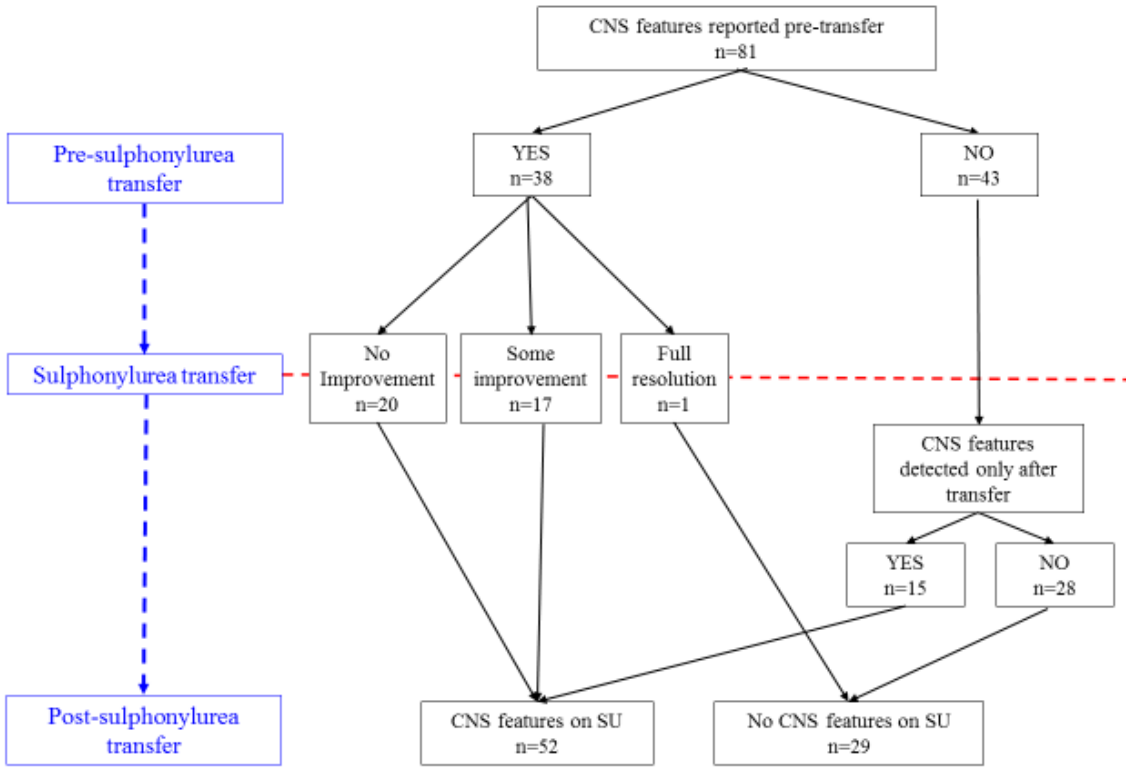

Figure 2C

Figure 2. Sulphonylurea Efficacy and Metabolic Control, and CNS Features. Panel A shows Kaplan Meier survival estimate of time to introduction of insulin in patients with sulphonylurea-treated *KCNJ11* PNDM. Panel B shows

longitudinal data for glycated haemoglobin values (primary y-axis) and sulphonylurea dose (secondary y-axis) in 74 patients on sulphonylurea without daily insulin at most recent follow-up (n=70 for pre-transfer glycated haemoglobin). Missing values were imputed by assuming a linear trend between available data points, carrying the last value forward or carrying the next value back. Panel C shows the number of patients for whom CNS features were reported before and after sulphonylurea transfer, and the number of patients who showed improvement of CNS features on sulphonylurea therapy.

Sulphonylurea therapy also remained safe. There were no reports of hypoglycaemia resulting in seizures or loss of consciousness in a total of 809 patient years of follow-up. This contrasts with the hypoglycaemia observed in 664 Norwegian patients with type 1 diabetes followed up for >8 years, with 296 patients (44.6%) reporting at least 1 episode of severe hypoglycaemia and 912 episodes reported in total.

Side-effects were reported by only 11/81 (13·6%) patients; 9 had gastrointestinal disturbance including 4 with transient diarrhoea, 1 with diarrhoea requiring further investigation, 2 with transient nausea, 1 with weight loss due to reduced appetite, and 1 with transient abdominal pain. One patient had initial hepatic steatosis and 1 had tooth discoloration. There were no reports of photosensitivity, hypersensitivity reactions, or abnormal renal function. No patients discontinued sulphonylurea treatment as a result of side-effects.

Daily insulin was required in addition to sulphonylureas in 6 patients at most recent follow-up (Table S2, Supplementary Appendix). Compared to patients

treated with sulphonylurea alone, patients also requiring insulin had worsened HbA1c at recent follow-up (HbA1c 8·5 [8·1-10·2]% (69.4 (65.0-88.0)mmol/mol) vs. 6·3 [5·9-7·1]% (41.0-54.1) mmol/mol), p=0.0006). A higher proportion in the insulin-treated group was male (40 of 75 vs. 6 of 6, p=0·03). Other characteristics were similar between the 2 groups (Table 1).

BMI decreased during follow-up, despite improved glycaemia: BMI SDS prior to sulphonylurea transfer vs. BMI at 10 years was (median[IQR]) 0·21(-0·25-0·84) vs. -0·25(-1·07-0·42), p=0·0009, n=58. Growth of Paediatric patients who remained independent of insulin over the period of follow-up was within the normal WHO reference range. Height SDS (median[IQR]) prior to sulphonylurea transfer and 10 years post-transfer (median[IQR]) was -0·46(-1·29-0·37) vs. -0·29(-1·01-0·73), p=0·31, n=38.

Diabetes complications were rare. Seven of 81 (8.6%) patients reported microvascular complications; retinopathy (n=5: 1 background, 2 non-proliferative, 1 pre-proliferative, 1 proliferative), microalbuminuria (n=2), proteinuria (n=1), and neuropathy (n=1). In 2 patients, complications developed prior to transfer to sulphonylureas. There were no macrovascular complications. Patients with complications were older at age of transfer to sulphonylureas than those without complications (age at transfer median[IQR] 20·5 [10·5-24·0] vs. 4·1 [1·3-10·2] years, p=0·0005). Other clinical characteristics were similar between the 2 groups (Table S3, Supplementary Appendix).

Table 1. Characteristics of Patients on Sulphonylurea Alone vs. Sulphonylurea and Insulin.

Characteristic	Patients Still on Sulphonylurea Without Daily Insulin (n=60-75)*	Patients Now on Insulin (+/- Sulphonylurea) (n=5-6)*	P Value (Patients on Sulphonylurea Alone vs. Patients on Insulin)§
KCNJ11 mutation	31 R201H, 18 V59M, 10 R201C, 2 G53D, 2 H46Y, 2 K170R, E51A, F33I, F35V, G53R, G53S, K170N, K170T, R201L, R50P, V59A	4 R201H, 1 R201C, 1 V59M	N/A
Age at sulphonylurea initiation (years), median(IQR)	4·3 (1·3-11·8) (n=75)	7·4 (4·7-10·5) (n=6)	0·36
Current age (years), median(IQR)	17 (13-23) (n=75)	19 (16-22) (n=6)	0·43
Male sex - % (number)	53 (40/75)	100 (6/6)	0·03
Birth weight (g), median(IQR)	2715 (2470-3040) (n=72)	2730 (2551-3120) (n=6)	0·71
Duration of follow-up (years), median(IQR)	10·2 (9·3-10·8) (n=75)	10·7 (9·7-11·2) (n=6)	0·39
Pre-sulphonylurea HbA1c (%), median(IQR)	8·0 (7·2-9·2) (n=70)	9·0 (8·9-9·7) (n=6)	0·12
Pre-sulphonylurea HbA1c (mmol/mol), median(IQR)	63.9 (55.2-77)	74.9 (73.8-82.5)	
Year 1 HbA1c (%), median(IQR)	5·9 (5·4-6·4) (n=66)	6·5 (6·2-6·6) (n=5)	0·06
Year 1 HbA1c (mmol/mol), median (IQR)	41 (35.5-46.4)	47.5 (44.3-48.6)	
Most recent HbA1c (%), median(IQR)	6·3 (5·9-7·1) (n=74)	8·5 (8·1-10·2) (n=6)	$6x10^{-4}$
Most recent HbA1c (mmol/mol),median (IQR)	45.4 (41-54.1)	69.4 (65-88)	
Pre-sulphonylurea insulin dose (U/kg/day), median (IQR)	0·68 (0·54-0·99) (n=66)	0·78 (0·70-0·80) (n=5)	0·58
Year 1 sulphonylurea dose (mg/kg/day), median(IQR)	0·30 (0·14-0·54) (n=68)	0·40 (0·25-0·52) (n=5)	0·58
Most recent sulphonylurea dose (mg/kg/day), median(IQR)	0·23 (0·12-0·45) (n=74)	0·27 (0·21-0·42) (n=6)	0·50
Pre-sulphonylurea BMI SDS	0·17 (-0·27-0·84) (n=60)	0·34 (-0·69-0·85) (n=5)	0·90
Most recent BMI SDS	-0·22 (-1·03-0·44) (n=72)	-0·40 (-0·72-0·06) (n=6)	0·74
Neurological features present at recent follow-up	65 (49/75)	50 (3/6)	0·46

Table 1. Characteristics of Patients on Sulphonylurea Alone vs. Sulphonylurea and Insulin. *n is different for each variable due to missing data. Year 1 values are those closest to the anniversary of the sulphonylurea transfer and had to fall between 3 months and 2 years for inclusion. Neurological features are defined as 1 or more of developmental delay, learning difficulties, sleep problems, ADHD, muscle weakness, epilepsy, anxiety, autism, or 'other' reported on by clinician.[§]Mann-Whitney test was used for numerical data, and test of proportions for categorical data. Abbreviations: BMI, body mass index; IQR, interquartile range; N/A, not applicable; SDS, standard deviation score

To evaluate beta-cell function, we performed oral and intravenous glucose-tolerance tests on sulphonylurea therapy in 6 patients 9·83 years (6·75-11·4) after transfer from insulin. Oral glucose-tolerance tests revealed a good insulin response to the glucose challenge (Figure 3). We observed a greater maximum insulin secretory response to oral than intravenous glucose (maximum insulin increment in response to oral glucose 69·6 pmol/litre [range 42·0-135·1] and in response to intravenous glucose 30·5 pmol/litre [range 0·0-46·9]) despite an increased plasma glucose stimulus. This suggests the increased incretin effect seen with sulphonylurea treatment after initial transfer [6] is well preserved after 10 years on sulphonylurea treatment.

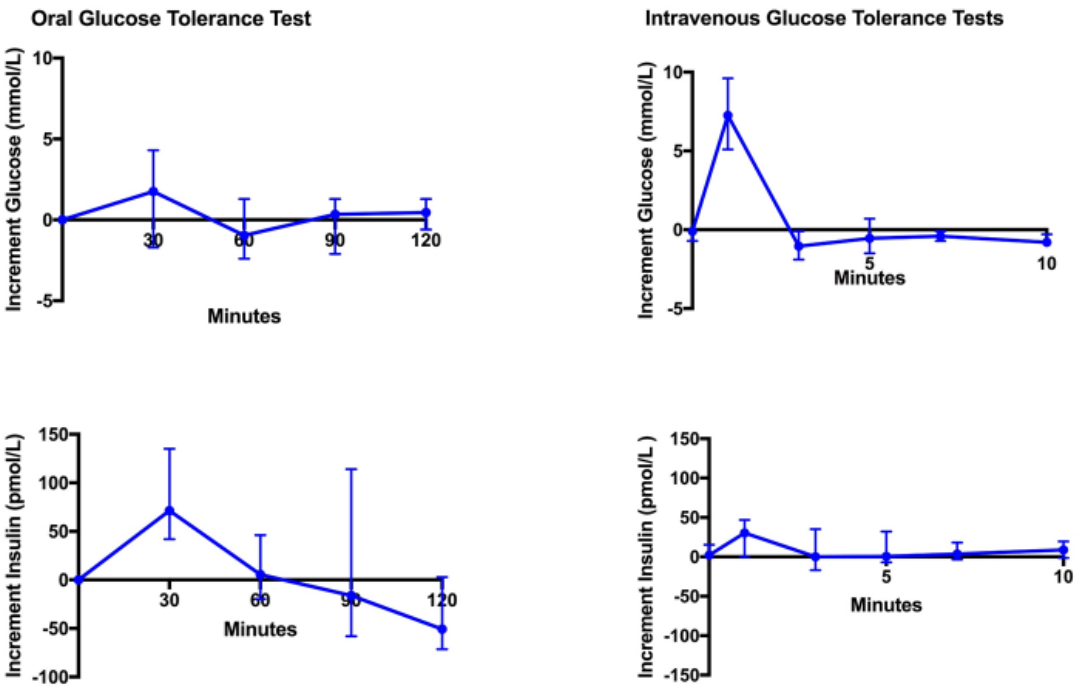

Figure 3. Physiological Studies.

Shown is median incremental increase in glucose and insulin concentration above baseline in an oral glucose tolerance test (6 patients) and an intravenous glucose tolerance test (6 patients). The test was performed after a median time of 9.83 years on sulphonylurea treatment.

CNS features were documented prior to transfer in 38/81 patients (Figure 2C, Table S4 Supplementary Appendix). Features were usually consistent with those previously described in patients with *KCNJ11* mutations but features associated with severe cerebral insult at the time of presentation with ketoacidosis were also present in 4 patients. All 17 patients with V59M, the commonest DEND-associated mutation, had CNS features prior to transfer (Table S4, Supplementary Appendix). There was an improvement reported in 18/38 patients at the time of sulphonylurea transfer, specifically in muscle tone (n=4), concentration/ADHD (n=5), gross motor skills (n=3), epilepsy (n=3), muscle weakness (n=3), learning difficulties (n=2), speech (n=1) and tics (n=1). However, improvement was incomplete in 17/18 patients and significant CNS

features remained. Full resolution occurred in only one female patient with unilateral hypotonia of the arm. At 10-year follow-up CNS features were seen in 52/81 patients, including 15 patients in whom CNS features were not noted prior to transfer to sulphonylureas (Table S4, Supplementary Appendix). These 15 patients transferred at median age 2.1 years and the new CNS features were mainly neuropsychological / psychiatric which would be more obvious as the child got older. Several additional neuropsychological / psychiatric features were also detected after transfer in those patients who had neurological involvement at baseline (Table S4, Supplementary Appendix).

DISCUSSION

In a large international cohort study of patients with *KCNJ11* PNDM, sulphonylurea therapy is effective and safe over 10 years. Over 90% of patients maintained excellent glycaemic control at long-term follow-up and were taking on average a lower dose of sulphonylurea when expressed as a dose per kilogram. This is consistent with previous studies, which followed single cases or much smaller cohorts of patients with *KCNJ11* PNDM over 2.5-5·7 years [8-10].

Our findings contrast markedly with type 2 diabetes where ~44% have inadequate glycaemic control despite increasing to a maximum dose after 5 years of treatment [7]. This difference probably reflects that: 1. in *KCNJ11* PNDM there is a fixed beta-cell defect which does not change over time while in type 2 diabetes there is a deterioration in beta-cell function after 1 year of ~5% per year [25], and 2. in *KCNJ11* PNDM high dose sulphonylureas facilitate the response to alternative pathway stimuli and are not directly stimulating insulin

secretion as they do in type 2 diabetes [6]. The prolonged action seen in *KCNJ11* PNDM also contrasts with many other examples of precision medicine where excellent initial results have not been maintained in the long term [12].

Excellent glycaemic control has been achieved without the usual side effects of hypoglycaemia and weight gain seen when intensive insulin therapy is used to improve glycaemic control [26]. The absence of any episodes of hypoglycaemia resulting in unconsciousness or seizures in over 800 patient years of follow-up is remarkable and in marked contrast to type 1 diabetes where intensive insulin treatment to improve control resulted in a ~3-times increase (16 vs. 5 episodes per 100 patient years) [27]. Most of our cohort were treated with glibenclamide which has been the sulphonylurea most associated with hypoglycaemia in type 2 diabetes [11]. The lack of severe hypoglycaemia is particularly reassuring as the doses of glibenclamide used in *KCNJ11* PNDM are ~4-10-times higher than those used in type 2 diabetes. In addition, our data shows a reduction in BMI of 0·46 SDS, p=0·0009 (~7% reduction in baseline adult equivalent BMI) over the 10-year follow-up period despite significantly improved metabolic control. In contrast, in the DCCT improved control was associated with ~4% increase in BMI over 1 year compared to conventional treatment [26]. Both lack of hypoglycaemia and lack of weight gain reflect that endogenous insulin secretion is tightly regulated in these patients.

Our extensive study has not found any unexpected side effects of high dose sulphonylurea therapy, but given that it only follows 81 patients, long-term surveillance of this cohort and other patients who transfer should continue to detect any unexpected side effects.

There are several possible reasons why 6/81 patients required daily insulin in addition to sulphonylurea therapy at follow-up. The median age at introduction

of insulin was 15 years, so many patients were peripubertal. This is important as puberty is associated with increased insulin resistance [28] and suboptimal treatment adherence in diabetes [29]. For 2 patients, poor adherence was specifically mentioned by their clinicians, and poor control usually continued even after insulin was added. Patients requiring re-introduction of insulin were on a relatively modest sulphonylurea dose (0·27 mg/kg/day, range 0·19-0·43), suggesting there was capacity to increase the dose further in all these. Taken together, our data suggest that other factors may have contributed to the need for additional daily insulin rather than sulphonylureas having stopped working at the level of the K_{ATP} channel.

We report low rates of diabetes-related complications in patients with *KCNJ11* PNDM. This likely reflects the improved glycaemic control that reduces micro-vascular complication risk as has been reported in type 1 diabetes [27]. The 8.6% of patients with complications transferred from insulin later than those without complications (20·4 vs. 4·1 years). Therefore, these had the suboptimal glycaemic control (8·7% pre-transfer) associated with insulin therapy for many years before the excellent control on sulphonylureas (6·5% post transfer). We propose therefore that the complications noted were largely the result of chronically elevated HbA1c prior to sulphonylurea transfer.

We demonstrate in physiological studies that the sulphonylurea-assisted insulin secretion shows a similar pattern after 10 years of follow-up as it did immediately post transfer [6]. Insulin secretion is excellent in response to oral glucose but is minimal in response to intravenous glucose reflecting that activating mutations in *KCNJ11* prevent the K_{ATP} channel from closing in response to metabolically generated ATP – a defect that is bypassed by sulphonylureas. Whilst the presence of sulphonylureas does increase the effect

of glucose-stimulated insulin secretion, this effect is small in comparison to the potentiation of insulin secretion seen in response to the incretins produced following a meal.

We report persistence of CNS features in *KCNJ11* PNDM despite long-term treatment with sulphonylureas, in contrast to the excellent glycaemic response. Although some initial improvement in CNS features was seen in 18/38 following sulphonylureas transfer, this was usually incomplete and subsequently plateaued. This initial improvement is consistent with the recent detailed prospective study by Beltrand et al [18]. Of note, a higher proportion of patients (64%) had CNS features reported at most recent follow-up than prior to transfer (47%). This may be explained by some patients having been too young to have had subtle features picked up clinically when first diagnosed, or heightened awareness amongst clinicians to look for subtle features at the most recent clinical follow-up as a result of improved characterisation of the CNS phenotype over the past decade.

The reason for the poor or absent CNS response despite an excellent long term glycaemic response in the same patients is uncertain. Both CNS features and diabetes are thought to be a direct result of the mutated channel, and sulphonylureas are likely to have a similar impact on the channels wherever their location. One possible explanation is that concentrations of glibenclamide in the CSF remain sub-therapeutic as a result of active transport across the blood brain barrier out of the brain [19]. Another possibility is that insulin secretion is supported by non-K_{ATP} channel mediated pathways which are not available for neuronal function [6] In addition, late transfer may have resulted in critical periods for brain development being missed; this is supported by the suggestion that earlier initiation of sulphonylurea treatment brings greater

benefit to neurological outcomes [18,30]. Further research is needed to investigate what treatment can improve CNS function in patients with *KCNJ11* mutations as our data shows this is a major clinical challenge for patients who now have excellent glycaemic control.

Our study has several strengths. To our knowledge, this is the largest cohort of people with *KCNJ11* PNDM to have been followed up, with 81 patients compared to 11 patients in the largest previous study [10]; it also represents the longest period of follow-up, greatly exceeding the 2.5-5.7 year follow up reported previously [8-10]. Ninety percent of eligible patients were included in the analysis, which is an excellent follow-up rate and ensures the findings accurately represent this unique population.

The study has limitations. Firstly, patients were not initially randomized to either sulphonylurea therapy or continuing on intensive insulin treatment so we cannot definitively rule out that the same outstanding outcome would not have been achieved on insulin therapy alone. However, these patients were insulin dependent and no long-term study of any type of insulin regime has produced long-term outcomes like these in type 1 diabetes. Secondly, the research involved multiple centres around the world, and there will have been variation in clinical practice in terms of type of sulphonylurea used, dosing of sulphonylurea, and threshold for reintroduction of insulin. However, the research reflects real-life clinical practice, and its multi-centre nature ensured that the largest possible number of patients was followed up, thereby increasing the power of the study and the generalisability of the findings. The main limitation of the evaluation of neurological features is the absence of a detailed neuropsychomotor assessment in one single centre before and after transfer in all patients.

Nevertheless, our data strongly supports the clinical experience of only a partial response of these features in some, but not all, affected patients.

Further work is required to establish efficacy and safety of sulphonylureas beyond 10 years and to investigate other aspects of treatment response such as effects of puberty. In addition, future research should further explore the impact of long-term sulphonylurea treatment on the neurological, neuropsychological and psychiatric features, through in-depth neuropsychomotor assessments repeated over time.

In conclusion, in a large international cohort of patients with *KCNJ11* PNDM, we have shown that sulphonylureas remain highly effective and safe when used for over 10 years. This supports the early and rapid genetic testing of infants with diabetes under 6 months of age, to facilitate prompt transfer of all patients with *KCNJ11* PNDM to sulphonylureas as an excellent long-term treatment.

DECLARATION OF INTEREST

We declare no competing interests.

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Supplementary Appendix

Lancet Diabetes and Endocrinology 2018 6(8): 637-646

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to Pamela Bowman, Åsta Sulen, Fabrizio Barbetti, Jacques Beltrand, Pernille Svalastoga, Ethel Codner, Ellen H. Tessmann, Petur Juliusson, Torild Skrivarhaug, Ewan R. Pearson, Sarah E Flanagan, Tarig Babiker, Nicholas J Thomas, Maggie H Shepherd, Sian Ellard, Iwar Klimes, Magda Szopa, Michel Polak, Dario Iafusco, Andrew T. Hattersley, Pål Rasmus Njølstad, the Neonatal Diabetes International Collaborative Group* Long-term treatment with sulphonylurea is highly effective and safe in neonatal diabetes due to *KCNJ11* mutations: an international cohort study

Contents

*Neonatal Diabetes International Collaborative Group

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Statistical Methods

Kaplan-Meier Analysis

All 81 patients were included in this analysis. For the 6 patients who had insulin reintroduced, time to reintroduction of insulin was calculated. For one patient in whom the date of reintroduction of insulin was not known, the most recent follow-up date was used to calculate duration and allow inclusion in the survival analysis. Patients who had not recommenced insulin were censored and their most recent follow up date was used in analysis. Number at risk at each year was plotted as shown in Figure 2A.

Data Imputation

Imputed data were generated by taking average values between 2 data points, assuming linear trends between data points and using equal increments depending on number of missing values, carrying the last value back or carrying the most recent value forward. Where 2 sets of data were available within a

given year, the data closest to the anniversary of the transfer were used and the other data were excluded.

Physiological Tests Repeated After 10 Years.

Oral Glucose Tolerance Test

Following an overnight fast and basal sampling, 1.75 g per kilo oral glucose (maximum 75 g) was ingested over 2 minutes at time 0 and plasma was collected at 0, 30, 60, 90 and 120 minutes for assay of glucose, insulin and c peptide. Sulphonylurea was taken as normal in the morning the day of the plasma collection.

Intravenous Glucose Tolerance Test

Following an overnight fast and basal sampling, 0.3 g per kg glucose was given over 1 minute and plasma was collected at -10, -4, 0, 1, 3, 5, 7, 10, 20, 60 minutes for glucose, insulin and c peptide assay. Sulphonylurea was taken as normal in the morning the day of the plasma collection. Increment was calculated as a delta value for each time point.

Laboratory Assays

Venous blood was obtained for centralized assays. Serum glucose and insulin were obtained before and after the glucose load. The Laboratory Clinic at Haukeland University Hospital, Norway, performed assays for glycated haemoglobin (HbA1c) with values aligned with those in the Diabetes Control and Complications Trial (DCCT) according to its standard procedures.

Table S1. Characteristics of Whole Cohort vs. Patients Eligible but Not Followed Up.			
Characteristic	**All Patients (N=65-81)***	**Patients Eligible but Not Included (N=3-8)***	**P Value[§]**
KCNJ11 mutation	35 R201H, 19 V59M, 11 R201C, 2 G53D, 2 H46Y, 2 K170R, E51A, F33I, F35V, G53R, G53S, K170N, K170T, R201L, R50P, V59A	2 R201H, 2 unknown, E22K, G53D, R201C, R50Q	N/A
Age at initiation of sulphonylurea treatment (years), median(IQR)	4.8 (1.7-11.4) (n=81)	15.0 (7.0-18.0) (n=8)	0.04
Age at diagnosis (weeks), median(IQR)	8.0 (4.0-12.0) (n=74)	0 (0-4.5) (n=8)	0.002
Male sex - % (number)	57 (46/81)	50 (4/8)	0.70
Birth weight (g)	2715 (2480 – 3050) (n=78)	2638 (2315-2875) (n=8)	0.36
Pre-sulphonylurea BMI SDS	0.18 (-0.28 – 0.84) (n=65)	0.51 (-0.83-1.91) (n=6)	0.63
Pre-sulphonylurea HbA1c (%), median(IQR)	8.2 (7.2-9.2) (n=76)	8.1 (7.4-8.3) (n=6)	0.70
Pre-sulphonylurea HbA1c (mmol/mol), median (IQR)	66.1 (55.2-77) (n=76)	65 (57.4-67.2) (n=6)	0.70
Year 1 HbA1c (%), median(IQR)	5.9 (5.5-6.5) (n=71)	6.2 (6.2-7.3) (n=5)	0.07
Year 1 HbA1c (mmol/mol), median (IQR)	41 (36.6-47.5) (n=71)	44.3 (44.3-56.3) (n=5)	0.07
Pre-sulphonylurea insulin dose (units/kg), median(IQR)	0.69 (0.59-0.94) (n=71)	0.68 (0.52-0.78) (n=8)	0.63
Year 1 sulphonylurea dose (mg/kg/day)	0.30 (0.14-0.53) (n=73)	0.53 (0.10-0.59) (n=5)	0.96

*N is different for each variable due to missing data. Year 1 values are those closest to the anniversary of the sulphonylurea transfer and had to fall between 3 months and 2 years for inclusion.
[§]Mann-Whitney test was used for numerical data, and 2 sample test of proportions for categorical data.
Abbreviations: BMI, body mass index; IQR, interquartile range; N/A, not applicable; SDS, standard deviation score.

Table S2. Characteristics of Patients Now on Daily Insulin and Sulphonylurea.*							
Case	**1**	**2**	**3**	**4**	**5**	**6**	**Median**
Current age	21	13	21	26	16	17	19
Age at sulphonylurea transfer (years)	10	3.2	10.5	14.3	4.7	4.8	7.4
Age at which insulin re-introduced (years)	15.3	12.6	18.3	22.5	n/a	12	15.3
Birth weight (g)	2551	2750	2450	3160	2710	3120	2730
Mutation	R201C	R201H	R201H	R201H	V59M	R201H	N/A
Duration of follow-up (years)	7.3	9.7	10.6	11.2	10.8	11.7	10.7
Duration when insulin re-introduced (years)	5.3	9.4	7.9	8.2	n/a	7.2	7.9
Most recent sulphonylurea dose (mg/kg/day)	0.43	0.42	0.23	0.31	0.21	0.19	0.27
HbA1c when insulin re-introduced (%)	9.2	8.1	11.4	7.7	n/a	n/a	8.7
HbA1c when insulin re-introduced (mmol/mol)	77	65	101.1	60.7	n/a	n/a	71.6
Most recent HbA1c on insulin (%)	8.8	n/a	12.9	8.2	10.2	7.7	8.5
Most recent HbA1c on insulin (mmol/mol)	72.7	n/a	117.5	66.1	88	60.7	69.4
Insulin name	Detemir	Glargine	Lispro and Glargine	Detemir	Detemir	Glargine	N/A
Most recent insulin dose (units/day)	26	31	21	8	91	12	23.5
Most recent insulin dose (units/kg/day)	0.45	0.75	0.32	0.13	1.89	0.22	0.38
Adherence* issues noted by clinician	No	Yes	Yes	No	No	No	N/A

*Adherence was not formally measured as part of the study, however 2 clinicians specifically reported poor adherence at some point during the follow up period.
Abbreviations: N/A, not applicable; n/a, not available.

Table S3. Characteristics of Patients With and Without Microvascular Complications.			
Clinical Characteristic	**Without Complications (N=59-74)***	**With Complications (N=6-7)***	**P Value[§]**
Age at sulphonylurea transfer (years)	4.1 (1.3-10.2) (n=74)	20.5(10.5-24.0) (n=7)	$5x10^{-4}$
Pre-sulphonylurea insulin dose (units/kg/day)	0.68 (0.57-0.94) (n=64)	0.82 (0.60-1.16) (n=7)	0.52
Pre-sulphonylurea HbA1c (%)	8.0(7.1-9.1) (n=69)	8.7(7.4-9.6) (n=7)	0.28
Pre-sulphonylurea HbA1c (mmol/mol)	63.9(54.1-76) (n=69)	71.6(57.4-81.4) (n=7)	0.28
Most recent HbA1c (%)	6.3(5.9-7.3) (n=73)	6.5(6.3-8.5) (n=7)	0.16
Most recent HbA1c (mmol/mol)	45.4(41-56.3) (n=73)	47.5(45.4-69.4) (n=7)	0.16
Pre-sulphonylurea BMI SDS	0.25(-0.32-0.89) (n=59)	0.17(0.02-0.24) (n=6)	0.63
Most recent BMI SDS	-0.24(-1.1-0.42) (n=71)	-0.24(-0.75-1.63) (n=7)	0.45

*N is different for each variable due to missing data. Complications were retinopathy (5 of 7), microalbuminuria (2 of 7), proteinuria (1 of 7), and neuropathy (1 of 7).
[§]Mann-Whitney test was used for numerical data
Abbreviations: BMI, body mass index; SDS, standard deviation score.

Table S4. Neurological Features				
Characteristic	**Neurological features identified before transfer to sulphonylureas (n=38)**		**Neurological features identified after transfer to sulphonylureas (n=15)**	**No neurological features identified (n=28)**
Mutation in *KCNJ11* gene	19 V59M, 7 R201H, 3 R201C, 2 G53D, 2 R50P, G53R, G53S, E51A, H46Y, V59A		6 R201H, 4 R201C, F33I, F35V, H46Y, K170N, R201L	21 R201H, 4 R201C, 2 K170R, K170T
Age at sulphonylurea initiation (years), median(IQR)	6.1 (1.3-12.1)		2.1 (0.4-8.9)	6.1 (2.8-12.5)
Current age (years), median(IQR)	18.0 (14.0-23.0)		14.0 (12.0-20.0)	19.0 (15.0-24.5)
Male sex - % (number)	55 (21/38)		60 (9/15)	57 (16/28)
Most recent sulphonylurea dose (mg/kg/day), median(IQR)	0.27 (0.12-0.50)		0.26 (0.14-0.36)	0.20 (0.13-0.38)
***KCNJ11* associated neurological features**	**Pre SU transfer (n)**	**Post SU transfer (n)**	**Post transfer only (n)**	N/A
Developmental delay (delayed developmental milestones)	29	26	0	
Epilepsy	10	11	1	
Muscle weakness	10	10	0	
Other*	12	7	0	
***KCNJ11* associated psychiatric/neuropsychological features**				N/A
Learning difficulties	18	30	14	
ADHD	4	12	5	
Autism	2	6	1	
Anxiety	2	10	4	
Sleep problems	2	3	2	
Other**	5	2	0	
Ketoacidosis related cerebral oedema associated CNS features				N/A
Spastic tetraplegia	3	3	0	
Spastic hemiplegia	1	1	0	

* Other features reported pre-transfer were hypotonia (5), cerebellar signs (5), gross/fine motor problems (2), myoclonic jerks (2), ptosis (1), tics (Tourette's) (1). Other features reported post transfer were hypotonia (1), cerebellar signs (2), gross/fine motor problems (2), ptosis (1), tics (Tourette's) (1).

**Other features reported pre-transfer attention or concentration deficits/hyperkinesis (5). Other features reported post-transfer attention or concentration deficits/hyperkinesis (2).

Abbreviations: ADHD, attention, deficit hyperactivity disorder; CNS, cerebral nervous system; IQR, interquartile range; N/A, not applicable; SU, sulphonylurea

CHAPTER 2

Patterns of post-meal insulin secretion in individuals with sulfonylurea-treated *KCNJ11* neonatal diabetes show predominance of non-K_{ATP}-channel pathways

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ATH, SEF and MHS contributed to conception and design of the study. KAP, BAK, ML, SRS, BMS, SEF, SH, MHS, RCA, TJM, and ATH contributed to data acquisition. KAP, BMS, SEF, MHS, RCA, TJM, and ATH contributed to analysis and interpretation of data. All authors critically revised the article. All authors approved the final version prior to submission.

MY CONTRIBUTIONS TO THE CHAPTER

I designed the study and developed the study protocol with ATH, SEF and MHS. I recruited the participants and undertook the physiology studies and sample collection with support from KAP, BAK, ML, SRS, BMS, SEF, SH, MHS, RCA, TJM, and ATH. I performed all data analyses and interpreted the findings with support from KAP, BMS, SEF, MHS, RCA, TJM, and ATH. I produced all figures and tables. I wrote the manuscript and revised it according to feedback from co-authors.

Main messages of the article

- Individuals with sulfonylurea-treated *KCNJ11* PNDM exhibit higher glucose values in response to a carbohydrate meal than to a protein meal, and for both meals glucose levels are higher than controls without diabetes, who exhibit much more tightly regulated glucose levels regardless of meal type.

- The more tightly regulated glucose levels in controls results from much higher insulin secretion in response to carbohydrate than to protein, whereas cases with sulfonylurea-treated *KCNJ11* PNDM have similar insulin levels in response to both meal types.

- The similar insulin secretion with the different meals in individuals with sulfonylurea-treated *KCNJ11* PNDM suggests a relative inability to modulate insulin secretion in response to both higher (carbohydrate) and lower (protein/fat) glucose levels, consistent with a dependence on non-K_{ATP} pathways for insulin secretion.

- Individuals with sulfonylurea-treated *KCNJ11* PNDM should avoid meals lacking carbohydrate or missing meals to mitigate the risk of post-prandial hypoglycemia.

Research questions remaining / emerging as a result of this work

- Do individuals with sulfonylurea-treated *KCNJ11* PNDM have intact glucagon secretion at low levels of glucose?

- Is the counter-regulatory response to hypoglycemia preserved in patients with sulfonylurea-treated *KCNJ11* PNDM?

- What is the effect of different doses and types of sulfonylurea on the physiological response to meals in individuals with *KCNJ11* PNDM?

Abstract

Objective

Insulin secretion in sulfonylurea-treated *KCNJ11* permanent neonatal diabetes (PNDM) is thought to be mediated predominantly through amplifying non-K_{ATP}-channel pathways such as incretins. Affected individuals report symptoms of post-prandial hypoglycemia after eating protein/fat-rich foods. We aimed to assess the physiological response to carbohydrate and protein/fat in people with sulfonylurea-treated *KCNJ11* PNDM.

Research Design and Methods

5 adults with sulfonylurea-treated *KCNJ11* PNDM and 5 age, sex and BMI-matched controls without diabetes had a high carbohydrate and high protein/fat meal on 2 separate mornings. Insulin(i) and glucose(g) were measured at baseline then regularly over 4 hours post-meal. Total area under the curve (tAUC) for insulin and glucose were calculated over 4 hours and compared between meals in controls and *KCNJ11* cases.

Results

In controls, glucose values after carbohydrate and protein/fat were similar (median glucose $tAUC_{0\text{-}4h}$ 21.4 vs.19.7mmol/L, p=0.08). In *KCNJ11* cases glucose levels were higher after carbohydrate than after protein/fat (median glucose $tAUC_{0\text{-}4h}$ 58.1 vs.31.3mmol/L, p=0.04). These different glycemic responses reflected different patterns of insulin secretion: in controls, insulin

secretion was greatly increased after carbohydrate vs. protein/fat (median insulin $tAUC_{0\text{-}4h}$ 727 vs.335pmol/L, p=0.04), but in *KCNJ11* cases insulin secretion was similar after carbohydrate and protein/fat (median insulin $tAUC_{0\text{-}4h}$ 327 vs.378pmol/L, p=0.50).

Conclusions

Individuals with sulfonylurea-treated *KCNJ11* PNDM produce similar levels of insulin in response to both carbohydrate and protein/fat meals despite carbohydrate resulting in much higher glucose levels and protein/fat resulting in relatively low glucose levels. This suggests an inability to modulate insulin secretion in response to glucose levels, consistent with a dependence on non-K_{ATP} pathways for insulin secretion.

Introduction

Activating *KCNJ11* mutations are the commonest cause of permanent neonatal diabetes (PNDM), diagnosed in the first 6 months of life (1). *KCNJ11* encodes Kir6.2, the pore-forming subunit of the ATP-sensitive potassium (K_{ATP}) channel that, in the beta-cell, closes in response to metabolically generated ATP, causing beta-cell depolarisation and insulin secretion. The SUR1 subunit, encoded by the *ABCC8* gene, further regulates K_{ATP} channel activity by opening in response to Mg-ADP, preventing insulin release (2). *KCNJ11* mutations impair the ATP sensitivity of pancreatic K_{ATP} channels rendering them unresponsive to rising blood glucose and the beta cell remains hyperpolarised (3), resulting in absolute insulin deficiency. Affected individuals required treatment with replacement doses of insulin until it was shown that sulfonylureas could bind and close pancreatic K_{ATP} channels resulting in beta-

cell depolarisation and endogenous insulin secretion (4). This allowed 90% of individuals with *KCNJ11* PNDM to stop insulin injections completely gaining excellent metabolic control which is maintained long-term (4, 5).

Severe hypoglycemia is rarely observed in sulfonylurea-treated *KCNJ11* PNDM (5, 6). This is remarkable given that the doses of sulfonylurea used in affected individuals are around 5-10 times those used to treat Type 2 diabetes (T2D) and indicates that sulfonylurea-stimulated K_{ATP} channel activity is regulated, at least in part, in the presence of *KCNJ11* mutations. However, as these mutations prevent regulation by ATP, it has been suggested that non-K_{ATP}-channel-mediated amplifying pathways of insulin secretion e.g. incretin hormones, predominate over the classical ATP pathway (4). This is supported by the very low levels of insulin secretion observed after intravenous glucose in comparison to oral glucose or meals, in individuals with sulfonylurea-treated *KCNJ11* PNDM (4). The role of sulfonylureas is assumed to be largely permissive in allowing the beta-cell to respond to non-K_{ATP}-channel-mediated amplifying pathways, as opposed to directly stimulatory (4).

Anecdotal reports from patients with *KCNJ11* PNDM have suggested mild-moderate hypoglycemia occurs after meals rich in protein/fat and lacking carbohydrate (7) or meals smaller than usual in size (6). This may reflect an inability to moderate food-stimulated insulin secretion in the context of falling glucose after a low carbohydrate meal; both GLP-1 and nutrient stimulation of the beta cell may play a role, as fatty acids and amino acids can drive insulin secretion through ATP-independent as well as ATP-dependent pathways (8-11). Similarly, K_{ATP} channels on pancreatic alpha cells (12) and glucose-sensing neurons in the brain ventromedial hypothalamus (VMH) (13) are thought to play a role in counter-regulatory responses to hypoglycemia via ATP-

dependent and independent mechanisms (14, 15). Sulfonylurea inhibition and protein can both stimulate alpha cell depolarisation and glucagon secretion *in vitro* (16, 17). However, counter-regulatory processes are complex and remain incompletely understood.

Despite its clinical importance in relation to dietary advice and hypoglycemia risk, no studies have investigated the impact of protein or other food types on insulin or glucagon secretion in sulfonylurea-treated *KCNJ11* PNDM. We therefore aimed to assess the insulin, glucose and glucagon response to carbohydrate and protein/fat in people with *KCNJ11* PNDM, and to compare this with the physiological response to the same food types in individuals without diabetes.

Research Design and Methods

Participants

5 adults >18 years of age with sulfonylurea-treated *KCNJ11* PNDM and 5 controls without diabetes matched for age, sex and BMI participated in the study. Clinical characteristics of the study participants are shown in Table 1. All measured characteristics were similar between the groups except fasting glucose which was higher in cases.

The study was approved by South West – Cornwall and Plymouth Research Ethics Committee and the Health Research Authority (REC reference 16/SW/0150). Written informed consent was obtained from all individuals prior to their participation. The study is registered on Clinicaltials.gov, identifier NCT02921906.

Clinical feature	***KCNJ11* Cases**	**Non-diabetic controls**	**p-value**
Age (years)	39.1 (24.4 – 41.0)	39.6 (24.2-41.8)	0.60
Sex male (%)	1 (20)	1 (20)	1.00
BMI	22.9 (22.4-26.8)	24.6 (23.9-25.6)	0.60
Fasting glucose (mmol/mol)	10.1 (8.6-11.9)	5.3 (4.8-5.5)	0.009
KCNJ11 mutation	4 R201H, 1 R201C	N/A	N/A
SU dose (mg/kg/day glibenclamide)	0.28 (0.07-1.21)	N/A	N/A
HbA1c (%)	6.9 (6.5-7.9)	N/A	N/A
HbA1c (mmol/mol)	52 (48-63)	N/A	N/A

Table 1. Clinical characteristics of study participants. All continuous numerical data are presented as median (range) unless otherwise stated. In one patient who was taking gliclazide and not glibenclamide, dose was converted to glibenclamide equivalent using % maximum dose according to British National Formulary.

Experimental Procedure

Participants attended the Exeter NIHR Clinical Research Facility and were given a high carbohydrate breakfast (77g carbohydrate, 9g protein, 1g fat, 371 calories) consisting of orange juice and white toast with jam, and a high protein/fat breakfast (46g protein, 18g fat, 6g carbohydrate, 369 calories) consisting of ham and cheese, on 2 separate mornings in a random order. Participants with *KCNJ11* PNDM took their usual prescribed dose of sulfonylurea (5 on glyburide, 1 on gliclazide) with each breakfast. These

individuals also took part in a third visit during which they did not have any breakfast but took their sulfonylurea tablet as usual that morning.

Before each visit the participants fasted overnight for a minimum of 10 hours. Prior to breakfast an IV cannula was inserted and 2 baseline blood samples (-5, 0 minutes) were taken for measurement of insulin, glucose, and glucagon. Breakfast was provided, on visits 1 and 2, and participants were given 15 minutes to eat. Participants were also given a glass of water and 1000mg paracetamol with the meal as a non-invasive measure of gastric emptying (11). Any food remaining after 15 minutes was removed and weighed. One control participant and one case did not finish the protein meal within 15 minutes (they ate 90% and 75% of the breakfast respectively). All participants ate the full carbohydrate meal. Blood samples were taken at regular intervals after breakfast (every 15 minutes for the first hour then every half hour for the last 3 hours); they were immediately centrifuged and frozen at -80^{o}C for later measurement of glucose, insulin, glucagon, and paracetamol levels. Participants were also screened at each time point for autonomic and neuroglycopenic symptoms of hypoglycemia using standard questions scored from 1-7 on a Likert scale as previously described (12, 13). For the individuals with *KCNJ11* PNDM, the third visit involved the same procedure but without any food.

Biochemical analysis

All biochemical analyses were performed in the Royal Devon and Exeter NHS Foundation Trust Clinical Laboratory. Serum glucose and paracetamol were analysed on the 702 module of the Cobas 8000 analyser, serum insulin was analysed on the 602 module of the Cobas 8000 analyser, and serum glucagon was analysed on the Dynex DS2 automated ELISA platform.

Statistical analyses

Data were analysed in Stata 14.2 using non-parametric statistical methods; Wilcoxon signed-ranks test for paired continuous data and Mann-Whitney test for unpaired continuous data (independent samples). For categorical data, Fisher's exact test was used for between group comparisons. Total area under the curve over 4 hours ($tAUC_{0\text{-}4h}$) and incremental area under the curve ($iAUC_{0\text{-}4h}$) for insulin, glucagon and glucose were calculated using the trapezoidal rule. Values are reported as median (range) throughout unless stated otherwise.

Glucose levels and hypoglycemia

Glucose trends after meals and responses to hypoglycemia questionnaires were described and glucose $tAUC_{0\text{-}4h}$ and $iAUC_{0\text{-}4h}$ were compared between meals in controls and *KCNJ11* cases.

Post meal insulin secretion

Insulin $tAUC_{0\text{-}4h}$ and $iAUC_{0\text{-}4h}$ were compared between meals in controls and *KCNJ11* cases. As baseline glucose values were different between cases and controls (Table 1, Supplementary figure 1), insulin was also adjusted for glucose by calculating ratios of total AUC for insulin and glucose (insulin tAUC/glucose tAUC)$_{0\text{-}4h}$ (18).

Post meal glucagon secretion

Glucagon $tAUC_{0\text{-}4h}$ and $iAUC_{0\text{-}4h}$ were compared between different meals in controls and *KCNJ11* cases.

Gastric emptying

Maximum serum paracetamol concentration (pmax) and time to maximum concentration (tmax) were used to calculate the emptying index (tmax / pmax),

which was compared between different meals to check for differences in rates of gastric emptying as previously described (19). Paracetamol $tAUC_{0\text{-}4h}$ was also compared between controls and *KCNJ11* cases and between meals.

Effect of sulfonylurea without food

To examine the effect of sulfonylurea alone, the glucose, insulin and glucagon analyses were repeated in *KCNJ11* cases after no food and compared to the responses to the carbohydrate and protein/fat meals.

Data cleaning

In the process of data analysis, for glucagon values <1.5 (limit of detection of the assay) a value of 1.4 was used. For paracetamol values <1.2 (limit of detection of the assay) a value of 1.1 was used. The baseline values for each analysis were an average of the minus-5-minute (-5m) and 0-minute (0m) values; if the -5m or 0m baseline value was missing, the single remaining baseline value was used. Where a value at a single time point was missing, an average of the values at the time points either side was imputed. On the 3 occasions where a sample was delayed due to the participant requiring recannulation, the result was allocated to the closest scheduled time point.

Results

Different glucose levels after protein/fat and carbohydrate in *KCNJ11* cases vs controls

Glucose levels were higher in cases vs. controls after both carbohydrate (glucose tAUC 58.1 (45.9-62.0) mmol/L vs. 21.4 (19.3-25.7) mmol/L, p=0.009) and protein/fat (glucose tAUC 31.3 (23.5 – 35.3) mmol/L vs. 19.7 (17.7-21.2) mmol/L, p=0.009), figure 1a.

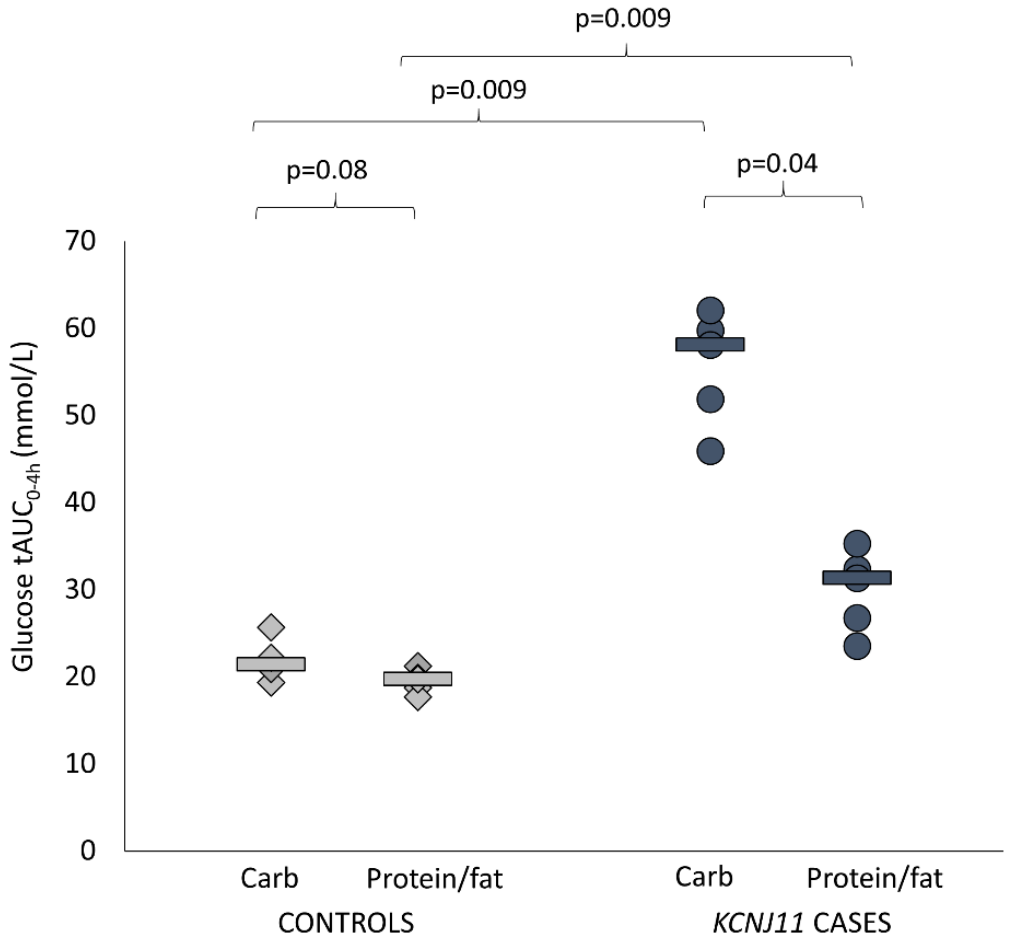

Figure 1a - Glucose total AUC over 4 hours. Controls are shown in light grey (diamonds are individuals and lines are group medians). KCNJ11 cases are shown in dark grey (circles are individuals and lines are group medians).

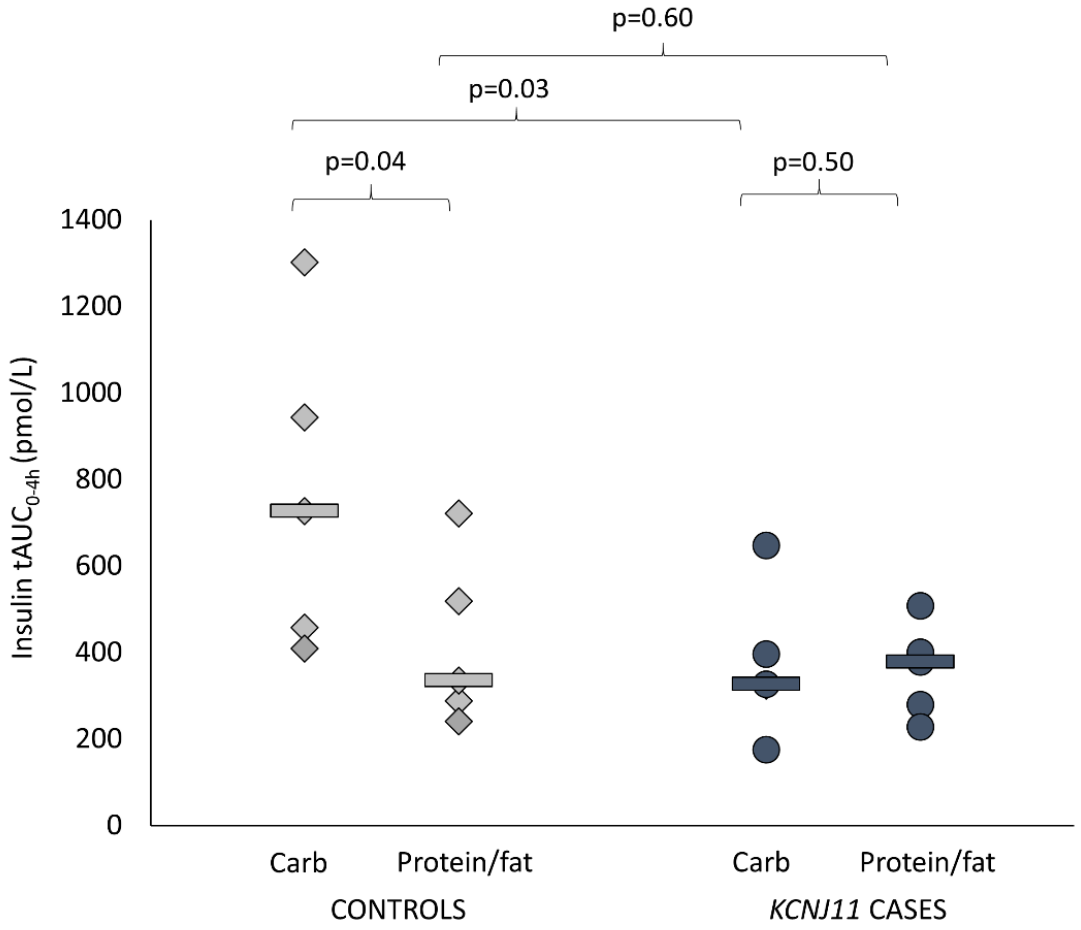

Figure 1b - Insulin total AUC over 4 hours. Controls are shown in light grey (diamonds are individuals and lines are group medians). KCNJ11 cases are shown in dark grey (circles are individuals and lines are group medians).

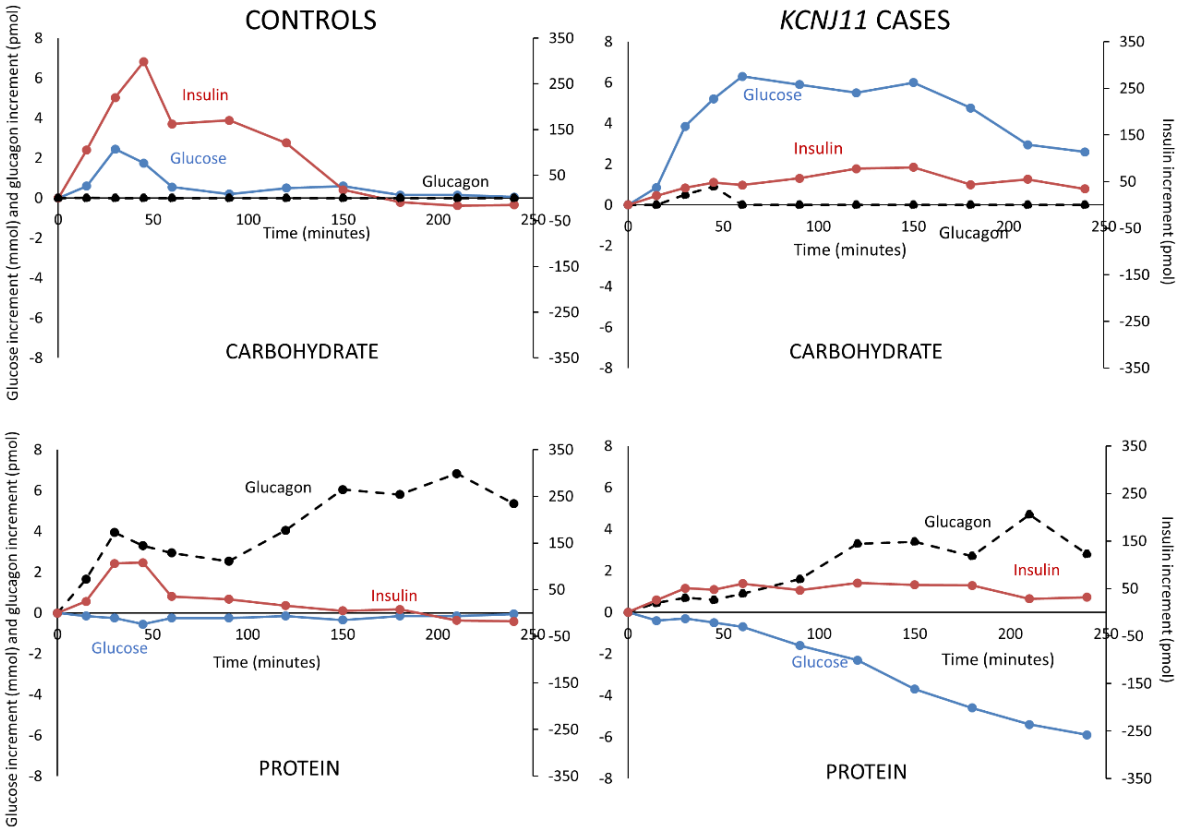

Figure 2 (a-d) – incremental glucose (blue solid line), insulin (red solid line) and glucagon (black broken line) in controls without diabetes and sulfonylurea-treated KCNJ11 cases with carbohydrate (upper panel) and protein/fat (lower panel) meals. Values shown are medians.

In controls, glucose values were tightly regulated after the 2 meals, figures 1a, 2 and Supplementary figure S1 (glucose tAUC after carbohydrate 21.4 (19.3-25.7) mmol/L and after protein/fat 19.7 (17.7-21.2) mmol/L, p=0.08). In *KCNJ11* cases glucose levels were much higher after carbohydrate (glucose tAUC after carbohydrate 58.1 (45.9-62.0) mmol/L than after protein/fat 31.3 (23.5 – 35.3) mmol/L, p=0.04), figures 1a, 2 and Supplementary figure S1. Similar trends were seen using iAUC (Supplementary table 1).

In cases, glucose increased 6.3 mmol/L from baseline in the first hour after carbohydrate and remained 2.6 mmol/L above baseline at 4 hours. In contrast, after protein/fat, glucose fell reaching median 5.9 mmol/L below baseline at 4 hours, figures 1 and S1. Despite falling glucose after protein/fat, no cases became hypoglycemic in the 4-hour study period: the lowest glucose value recorded was 4.4mmol/L. Consistent with these glucose profiles, all cases and controls had the lowest possible scores at all time points when screened for symptoms of hypoglycemia, indicating an absence of subjective symptoms. There were also no objective symptoms of hypoglycemia during observation of participants by the study nurse and doctor.

Different glycemic responses to meals were explained by different patterns of insulin secretion in *KCNJ11* cases vs controls

Insulin secretion was higher in controls vs cases after the carbohydrate meal (insulin $tAUC_{0\text{-}4h}$ 727 (409-1302) vs. $tAUC_{0\text{-}4h}$ 327(175-647) pmol/l, p=0.03) but not after the protein/fat meal (insulin $tAUC_{0\text{-}4h}$ 335(241-722) vs. 378(228-508) pmol/l p=0.60), figure 1b. In controls insulin secretion was greatly increased after the carbohydrate meal compared to the protein/fat meal (insulin $tAUC_{0\text{-}4h}$ 727 (409-1302) vs 335(241-722) pmol/l, p=0.04, but in the *KCNJ11* cases insulin secretion was similar with the 2 meals (insulin $tAUC_{0\text{-}4h}$ 327(175-647) after carb and 378(228-508) pmol/l after protein/fat) p=0.50, Figures 1b, 2 and Supplementary figure 1.

The same pattern was observed using iAUC (Supplementary table 1) and when insulin secretion was adjusted for glucose (insulin tAUC/glucose tAUC)$_{0\text{-}0.4h}$ in controls after carbohydrate vs protein/fat, 33.9 (19.6-50.8) vs 18.9 (11.4-36.2),

p=0.04 and in *KCNJ11* cases 6.2 (2.9-14.1) vs 11.7 (9.7-14.4), p=0.08), Supplementary figure 2. These results are consistent with *KCNJ11* cases not being glucose responsive, in contrast to controls without diabetes.

Glucagon secretion is increased in response to protein/fat compared to carbohydrate in both cases and controls

Both controls and cases had higher glucagon secretion after protein/fat than carbohydrate (glucagon $tAUC_{0\text{-}4h}$ in controls 32.5 (13.8-37.9) vs 7.2 (5.6-11.2) pmol/l, p=0.04 and in cases 17.4 (7.8-28.3) vs 6.1 (5.7-8.9) pmol/l, p=0.04), Figures 2, 3 and Supplementary figure 1. This is consistent with an alpha cell response to amino acids and / or fatty acids in both groups.

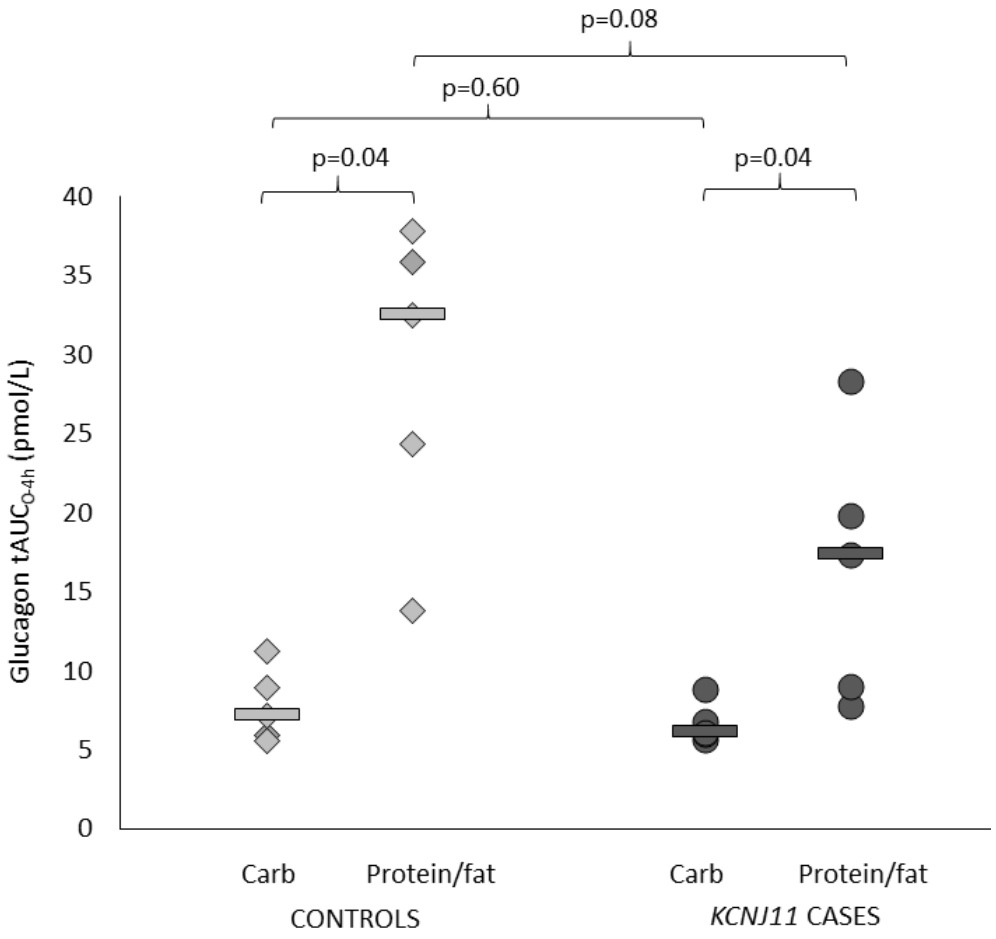

Figure 3 - Glucagon total AUC over 4 hours. Controls are shown in light grey (diamonds are individuals and lines are group medians). KCNJ11 cases are shown in dark grey (circles are individuals and lines are group medians).

Paracetamol profiles indicate similar rates of gastric emptying

Paracetamol concentration-time curves are shown in Supplementary figure 3. Emptying index (tmax/pmax) and tAUC were similar between controls vs cases (tmax/pmax for carbohydrate 0.03 vs 0.05, p= 0.46 and for protein/fat 0.04 vs 0.03, p=0.60, paracetamol $tAUC_{0\text{-}4h}$ for carbohydrate 30.5 (21.9-55.5) vs 28.5 (18.5-44.4) mg/L, p=0.75 and for protein/fat 28.4 (24.4-46.7) vs 36.5 (13.6-40.4), p=0.92) mg/L. Emptying index and tAUC were also similar between meals in controls (emptying index p=0.89, tAUC0-4h p=0.45) and in cases (tmax/pmax p=0.50, tAUC0-4h p=0.92).

Effects of sulfonylurea independent of food in *KCNJ11* cases

Glucose fell to a similar extent after both sulfonylurea only (no food) and protein/fat (Supplementary figures 4&5) and there was no difference in overall glucose levels (glucose tAUC0-4h with no food 34.6 (28.7-42.8) mmol/L vs and after protein/fat 31.3 (23.5 – 35.3) mmol/L, p=0.22). In contrast, glucose levels were lower after sulfonylurea only vs carbohydrate ((glucose $tAUC_{0\text{-}4h}$ with no food 34.6 (28.7-42.8) vs 58.1 (45.9 – 62.0) mmol/L, p=0.04). The same trends were seen using glucose iAUC (Supplementary table 1).

Insulin secretion in the 4 hours after a meal was higher after carbohydrate vs. sulfonylurea only (insulin tAUC0-4h 327(175-647) vs. 174(129-280) pmol/l,

p=0.04, and insulin iAUC 205(104-480) vs. 39(13-101) pmol/l, p=0.04). There was also a trend towards higher insulin secretion after protein/fat vs. sulfonylurea only (insulin tAUC0-4h 378(228-508) vs. 174(129-280) pmol/l, p=0.08, and insulin iAUC 183(109-316) vs. 39(13-101) pmol/l, p=0.04), Supplementary figures 4&5. This supports a key role for food in triggering insulin release in the context of sulfonylurea-treated *KCNJ11* PNDM.

Glucagon secretion was similar in the absence of food vs. carbohydrate (glucagon $tAUC_{0\text{-}4h}$ 6.0 (5.6-18.7) vs 6.1 (5.7-8.9) pmol/l, p=0.69, and glucagon iAUC 0.2(-0.2-1.9) vs 0.5(0.1-1.2) pmol/l, p=0.50)) despite very different glucose levels. In contrast, there was a trend towards lower glucagon secretion in the absence of food vs. protein/fat (glucagon $tAUC_{0\text{-}4h}$ 6.0 (5.6-18.7) vs 17.4 (7.8-28.3) pmol/l, p=0.22, and glucagon $iAUC_{0\text{-}4h}$ 0.20 (-0.2-1.9) vs 11.8 (2.2-16.7) pmol/l, p=0.04).

The rate of gastric emptying did not change with sulfonylurea only in comparison to the two meals (paracetamol tAUC0-4h with no food 34.6 (20.0-43.4) mg/L and with carbohydrate 28.5 (18.5-44.4) mg/L, p=0.89, paracetamol $tAUC_{0\text{-}4h}$ with no food 34.6 (20.0-43.4) mg/L and with protein/fat 36.5 (13.6-40.4) mg/L, p=0.69).

Discussion / Conclusions

We have shown clear differences in glucose levels and insulin secretion after carbohydrate and protein/fat meals between controls without diabetes and individuals with sulfonylurea-treated *KCNJ11* PNDM.

Whilst controls show tightly regulated glucose levels after meals, individuals with *KCNJ11* PNDM have lower glucose after protein/fat vs. carbohydrate.

These different glucose profiles have clinical implications in terms of dietary advice offered to patients with sulfonylurea-treated *KCNJ11* PNDM. In this specific type of diabetes, patients should avoid meals or diets completely lacking carbohydrate since blood glucose is likely to fall post-prandially in the context of a protein/fat meal. Furthermore, they should avoid missing meals after taking sulfonylurea, as we also observed a fall in glucose with sulfonylurea in the absence of food.

In addition to the practical implications, our data may provide a mechanistic explanation as to why patients with sulfonylurea-treated *KCNJ11* PNDM report hypoglycemia after protein/fat-rich meals. Insulin secretion after protein/fat and carbohydrate is similar in affected individuals despite very different glucose profiles. This supports insensitivity to glucose and lack of moderation of incretin and amino acid/fatty acid-stimulated insulin secretion after a protein/fat meal. Consistent with this is human and rodent data from previous studies demonstrating non-K_{ATP}-driven insulin secretion in the presence of normal or low glucose. Humans with congenital hyperinsulinism (CHI) caused by recessively inherited inactivating (loss of function) K_{ATP} channel mutations have insensitivity to leucine, which acts through K_{ATP} pathways, but sensitivity to glutamine, which acts independently of ATP to drive insulin secretion and hypoglycemia after protein-rich meals (20). Furthermore, SUR1 knockout mice are euglycemic but show amino acid-stimulated insulin secretion which is particularly sensitive to glutamine (21).

The pattern of the insulin response to the different meals in sulfonylurea-treated *KCNJ11* PNDM contrasts with the pattern in controls without diabetes where carbohydrate, acting through the classical ATP pathway, elicits a far greater insulin response than protein/fat and is quickly ‘switched off’ in response to

normalisation of blood glucose. It also contrasts with the response in individuals with Type 1 diabetes on intensive insulin therapy, who show rises in post-prandial glucose 3-5 hours after a high protein/fat meal and in whom protein is protective against hypoglycemia (22).

Our results are consistent with the previously hypothesised mechanism of insulin secretion in patients with sulfonylurea-treated *KCNJ11* PNDM. Pearson et al. reported a large reduction in insulin secretion following intravenous glucose vs oral glucose supporting predominance of non-K_{ATP} mediated amplifying pathways over the classical ATP pathway in these patients (4). The small amount of insulin secretion and fall in glucose we observed in the absence of food contrasts with the idea of a purely permissive action of sulfonylureas on the beta-cell (4), although our experimental design differed from previous physiological studies limiting direct comparison. The very high doses of sulfonylurea used to treat *KCNJ11* PNDM do not result in severe hypoglycemia highlighting the possibility of a different pharmacological mechanism to the direct effects on the K_{ATP} channel seen in Type 2 diabetes. Further research is needed to improve understanding of the mechanism of sulfonylurea action in *KCNJ11* PNDM.

In our study, glucagon secretion was higher after protein/fat vs carbohydrate in both cases with *KCNJ11* PNDM and controls without diabetes, consistent with the previously described stimulatory effect of amino acids on alpha cells (17). We did not test the glucose-responsiveness of alpha cells in this study as all individuals remained euglycemic throughout. However, rodent models suggest defective glucagon secretion may occur in the presence of K_{ATP} channel mutations. Specifically, SUR1 knockout mice show an alpha cell secretory defect at low levels of glucose (23), *KCNJ11* knockout mice exhibit defective

glucagon secretion due to an impaired brain response to hypoglycemia (24), and intracerebroventricular perfusion of K_{ATP} channels with SU in conscious rats reduces glucagon responses to hypoglycemia (25). In humans, the research and anecdotal clinical evidence to date suggests that these patients are protected from severe hypoglycemia, but the mechanisms of this remain unknown and future research will investigate in detail the counter-regulatory response to hypoglycemia in people with *KCNJ11* mutations at the level of the alpha cell and the brain as previously described in the context of glucokinase mutations (26).

Our study has important strengths. To our knowledge, it is the first study to assess the impact of different food types on glucose levels and insulin and glucagon secretion in people with *KCNJ11* PNDM, and to compare this with data from controls without diabetes. Previous research has been limited to assessment of insulin secretion following oral and intravenous glucose tolerance tests in small groups of affected individuals without a control group for comparison (4, 5).

Our study has some limitations. Firstly, the numbers of cases and controls are small and only 2 mutations in the *KCNJ11* gene (R201H and R201C) were studied, reflecting the rarity of the disease. However, these are the most common mutations and both impact ATP binding. Secondly, as the individuals in our study did not have glucose levels in the hypoglycemic range, we were unable to assess the alpha cell response in the presence of low glucose as discussed above. Finally, the study was only done in adults, which may limit the generalisability of the findings, particularly for patients in the paediatric age range. Additional studies in children with sulfonylurea-treated *KCNJ11* PNDM will be required to investigate the beta and alpha cell responses to carbohydrate

and protein/fat and to establish whether these responses differ to those observed in adults.

In conclusion, we have shown that individuals with sulfonylurea-treated *KCNJ11* PNDM produce similar levels of insulin in response to both carbohydrate and protein/fat meals despite carbohydrate meals resulting in much higher glucose levels and protein/fat meals being characterized by relatively low glucose levels. This suggests an apparent inability to modulate insulin secretion in response to both higher (carbohydrate) and lower (protein/fat) glucose levels, which is consistent with a dependence on non-K_{ATP} pathways for insulin secretion. Our findings may provide a mechanistic explanation for the post-prandial hypoglycemia reported by patients with *KCNJ11* PNDM. Furthermore, glucose levels can fall with sulfonylureas in the absence of food. We would therefore recommend that affected individuals avoid missed meals or meals lacking carbohydrate whilst on sulfonylurea treatment. Finally, our data highlights the utility of *KCNJ11* PNDM as a model for studying non-K_{ATP}-mediated pathways of insulin secretion and demonstrates the predominance of the classical ATP pathway in the non-diabetic state.

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guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Conflicts of interest disclosures: None to declare.

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Supplementary Material

BMJ Open Diabetes Research and Care 2019;7:e000721. doi: 10.1136/bmjdrc-2019-000721

Outcome	Group	Carb	Protein	No food	P-value carb vs protein	P-value protein vs no food	P-value carb vs no food
Glucose iAUC (mmol/L)	Cases	16.7 (8.3-31.6)	-10.9 (-29.1 - -2.2)	-7.6 (-11.8-3.9)	0.04	0.22	0.04
	Controls	1.00 (-0.7-3.3)	-1.15 (-1.5- -0.9)	N/A	0.04	N/A	N/A
Insulin iAUC (pmol/L)	Cases	205 (104-480)	183 (109-316)	39 (13-101)	0.69	0.04	0.04
	Controls	472 (230-992)	70 (8-310)	N/A	0.04	N/A	N/A
Glucagon iAUC (pmol/L)	Cases	0.5 (0.1-1.2)	11.8 (2.2-16.7)	0.2 (-0.2-1.9)	0.04	0.04	0.50
	Controls	0.3 (-2.8-1.7)	18.4 (-6.9-26.3)	N/A	0.14	N/A	N/A

Supplementary Table 1. Incremental area under the curve (iAUC) for glucose, insulin and glucagon after different meals in KCNJ11 cases and controls.

iAUC = incremental AUC over 4 hours

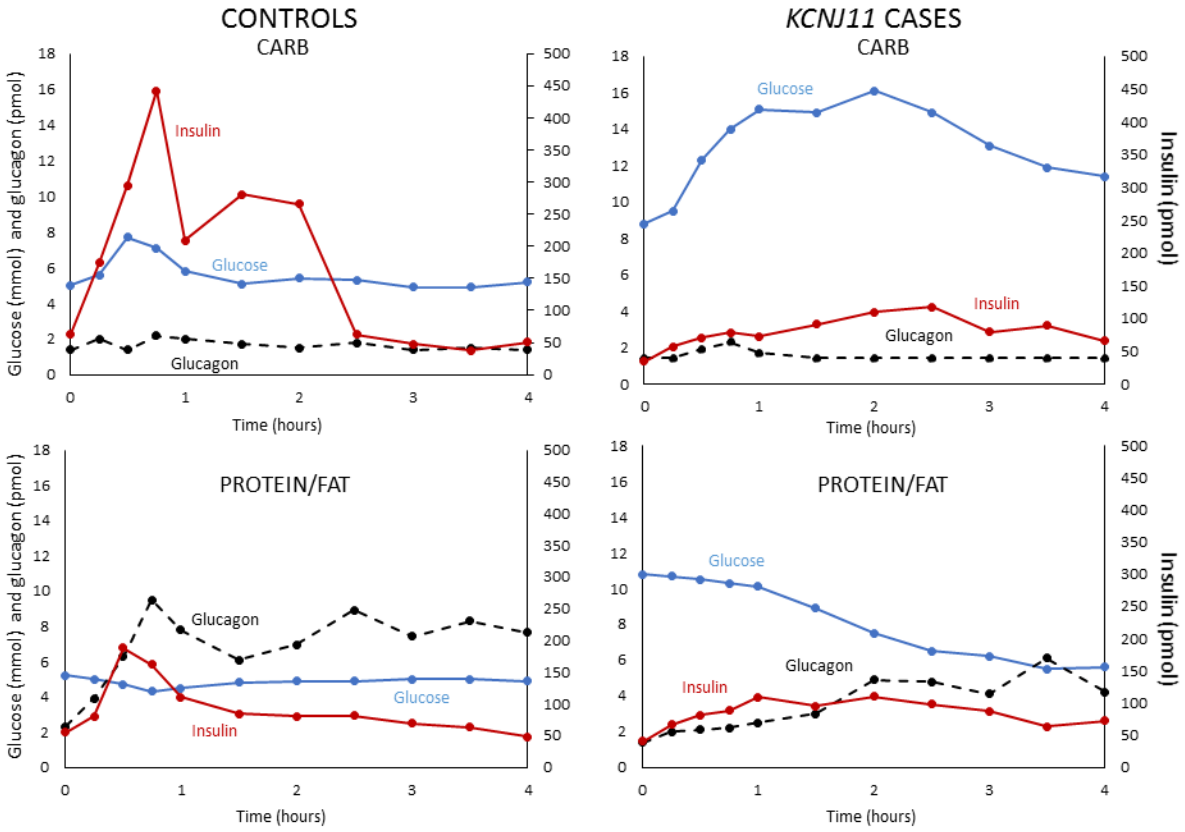

Figure S1 – absolute values insulin, glucagon and glucose in controls without diabetes and KCNJ11 cases in response to carbohydrate and protein meals. Values shown are medians.

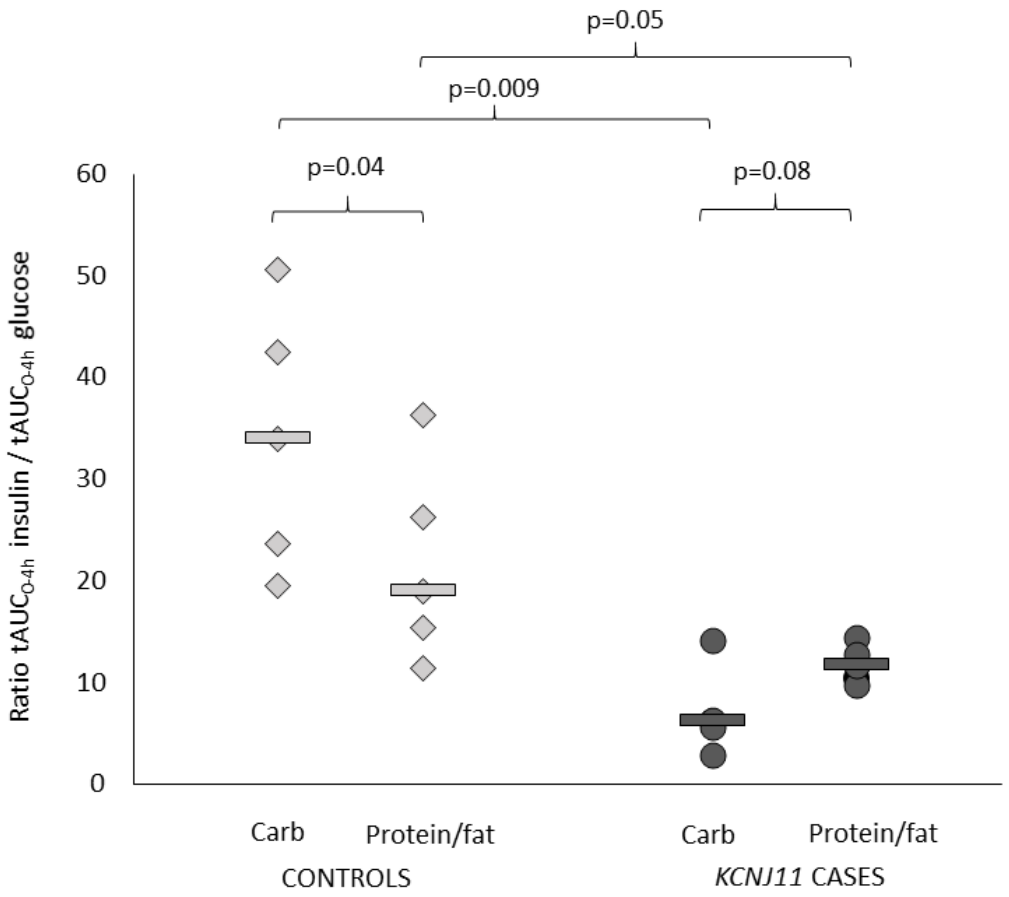

Figure S2 - ratio of total AUC0-4h insulin / total AUC0-4h glucose. Controls are shown in grey (diamonds are individuals and lines are group medians). KCNJ11 cases are shown in black (circles are individuals and lines are group medians).

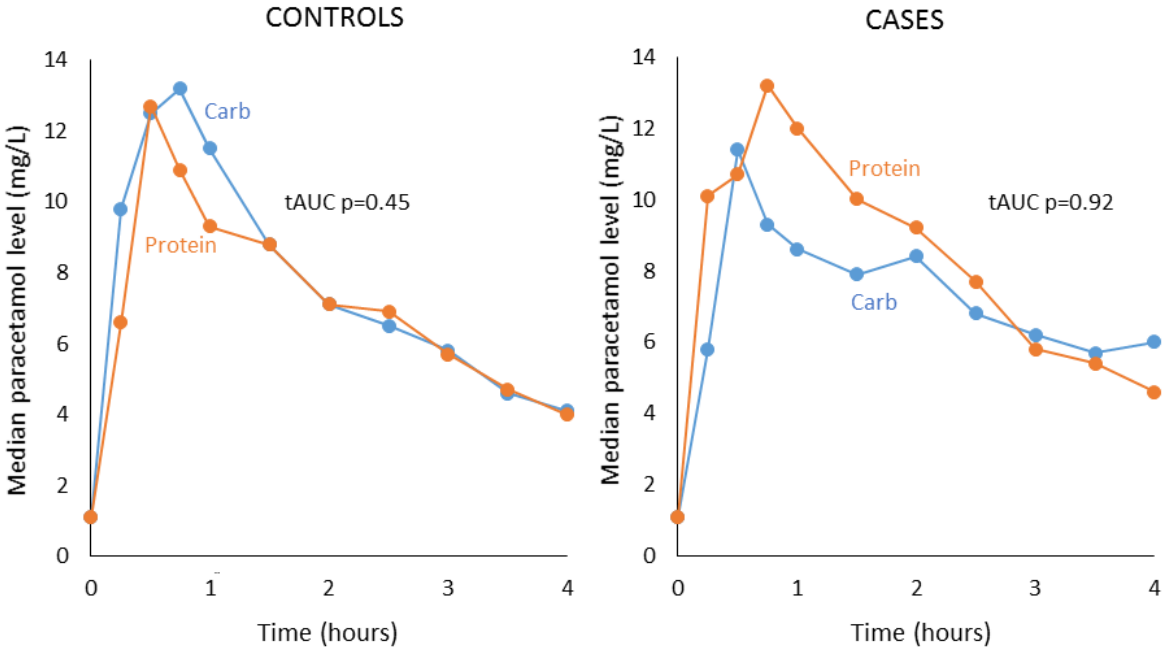

Figure S3 – paracetamol absorption curves in controls and cases with the carbohydrate (blue) and protein/fat (orange) meals.

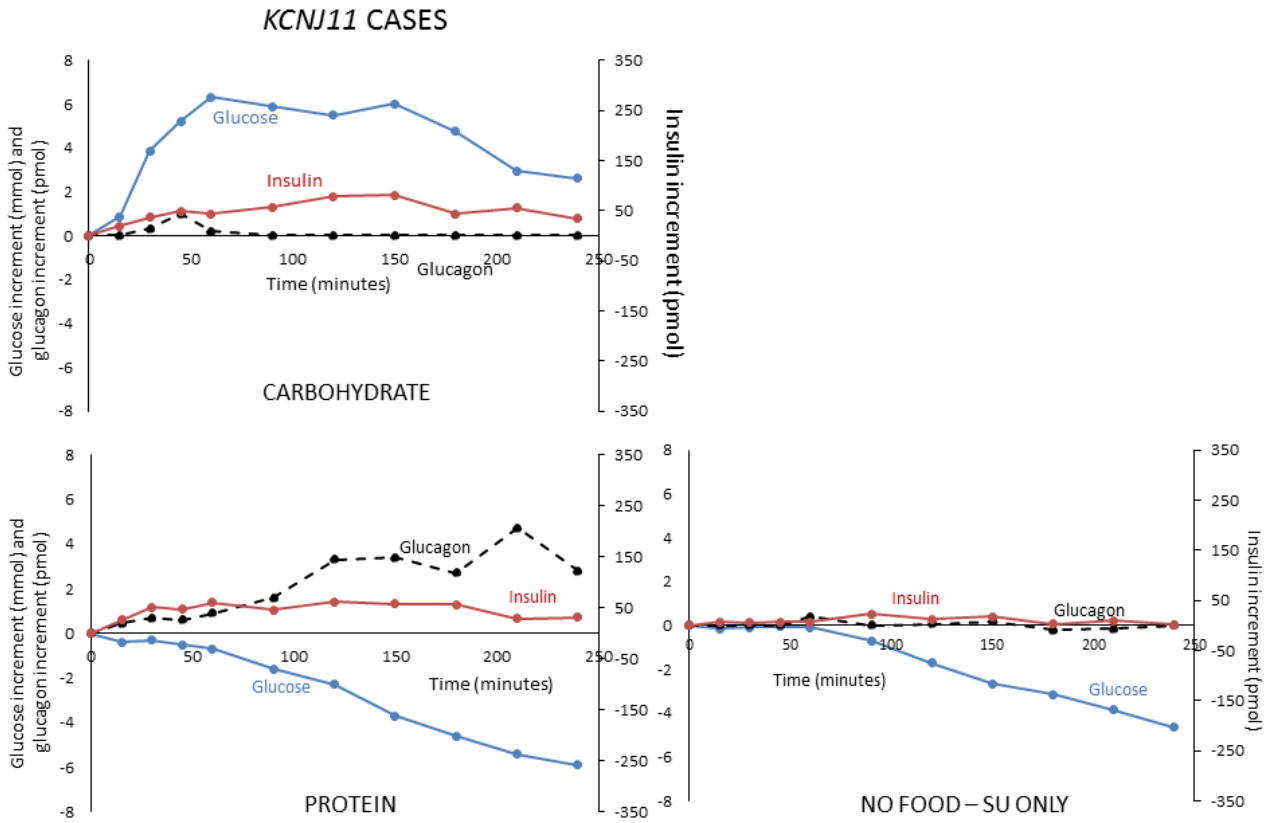

Figure S4 – incremental glucose, insulin and glucagon in KCNJ11 cases with sulfonylurea only in the absence of food in comparison to the carbohydrate and protein/fat meal. Values shown are medians.

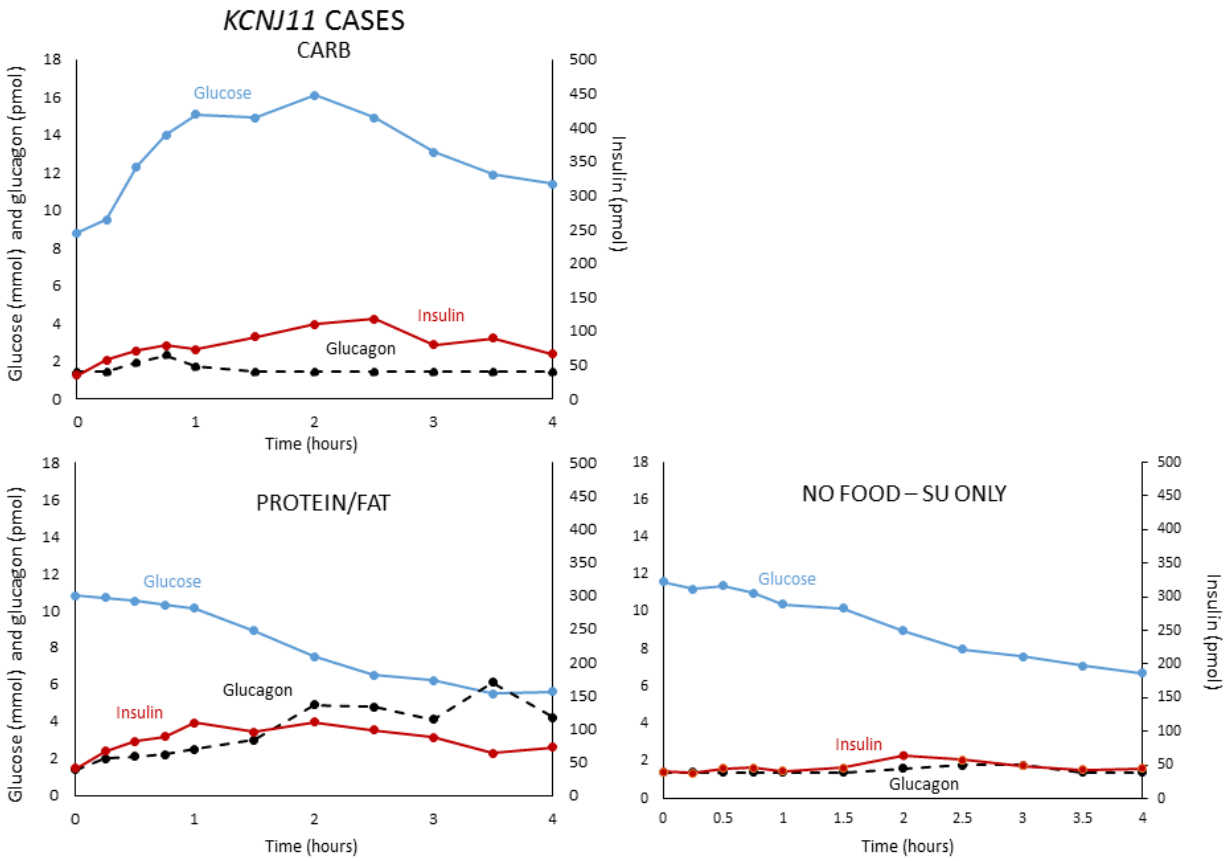

Figure S5 - absolute glucose insulin, and glucagon and in KCNJ11 cases with sulfonylurea only in the absence of food in comparison to the carbohydrate and protein/fat meal. Values shown are medians.

CHAPTER 3

PART A

Psychiatric morbidity in children with *KCNJ11* neonatal diabetes

Pamela Bowman, Emily Broadbridge, Bridget A. Knight, Lewis Pettit, Sarah E. Flanagan, Marie-Claire Reville, James Tonks, Maggie H. Shepherd, Tamsin J. Ford, Andrew T. Hattersley

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ACKNOWLEDGEMENTS / CONTRIBUTIONS

ATH, PB, TJF and JT contributed to conception and design of the study. PB, TJF, BK, LP, MS, JT and MR contributed to data acquisition. PB, TJF, ATH, SEF, MS and EB contributed to analysis and interpretation of data. PB, TJF and ATH drafted the article and all other authors critically revised it. All authors approved the final version prior to submission.

MY CONTRIBUTIONS TO THE CHAPTER

I designed the study and developed the protocol with ATH, TJF and JT. I recruited participants and their parents and teachers and collected data through the administration of the SDQ and DAWBA questionnaires, with support from TJF, BK, LP, MS, JT and MR. I undertook clinical rating of the DAWBA questionnaires with TJF. I performed all data analyses and interpreted the findings with support from TJF, ATH, SEF, MS and EB. I provided feedback on the study findings to participants with support from TJF and JT. I produced all figures and tables. I wrote the manuscript and revised this according to feedback from co-authors.

NOVELTY STATEMENT

- This is the first study to systematically assess psychiatric morbidity in people with *KCNJ11* mutations, using validated, standardised diagnostic tools.
- The data show that *KCNJ11* mutations, in addition to causing neonatal diabetes, also cause psychiatric disorders that are clinically unrecognised but have high impact on families.
- This research highlights the need for early assessment and an integrated and collaborative approach to clinical care in people with *KCNJ11* neonatal diabetes.

ABSTRACT

Aims

Mutations in the *KCNJ11* gene, which encodes the Kir6.2 subunit of the pancreatic K_{ATP} channel, cause neonatal diabetes. *KCNJ11* is also expressed in the brain, and approximately 20% of those affected have neurological features, which may include features suggestive of psychiatric disorder. No previous studies have systematically characterised the psychiatric morbidity in people with *KCNJ11* neonatal diabetes. We aimed to characterise the types of psychiatric disorders present in children with *KCNJ11* mutations, and explore their impact on families.

Methods

The parents and teachers of 10 children with neonatal diabetes due to *KCNJ11* mutations completed the Strengths and Difficulties Questionnaire (SDQ) and DAWBA (Development and Wellbeing Assessment). SDQ scores were compared with normative data. Diagnoses from the DAWBA were compared with known clinical diagnoses.

Results

SDQ scores indicated high levels of psychopathology and impact. Psychiatric disorder(s) were present in all 6 children with the V59M or R201C mutation, and the presence of more than one psychiatric disorder was common. Only 2 children had received a formal clinical diagnosis, with a further one awaiting assessment, and coexistence of more than one psychiatric disorder had been missed. Neurodevelopmental (attention deficit hyperactivity disorder and autism) and anxiety disorders predominated.

Conclusions

Systematic assessment using standardised validated questionnaires reveals a range of psychiatric morbidity in children with *KCNJ11* neonatal diabetes. This is under-recognised clinically and has a significant impact on affected children and their families. An integrated collaborative approach to clinical care is needed to manage the complex needs of people with *KCNJ11* neonatal diabetes.

INTRODUCTION

Mutations in *KCNJ11*, which encodes the Kir6.2 subunit of the K_{ATP} channel, are the commonest cause of neonatal diabetes. These are important to diagnose as over 90% of people with these mutations can transfer from insulin treatment to an oral sulphonylurea, achieving excellent glycaemic control (1). *KCNJ11* is expressed in the brain as well as the pancreas (2), explaining why approximately 20% of people with mutations in this gene have a neurological phenotype known as DEND (Developmental delay, Epilepsy and Neonatal Diabetes) syndrome (3). Even those without an overt neurological phenotype have recently been shown to have attention deficits and developmental coordination disorder on neuropsychological testing (4).

Case reports and animal data suggest that *KCNJ11* mutations may be associated with childhood psychiatric disorders. Two people with the commonest DEND mutation, V59M, have been reported to have attention deficit hyperactivity disorder (ADHD) (5). Mouse models with V59M mutations targeted to neuronal tissue have replicated the hyperactive phenotype, and show increased exploratory behaviour and reduced anxiety behaviour consistent with the inattention and impulsivity reported in humans, suggesting a role for *KCNJ11* in emotional regulation (6). Autism (comprising impaired language and social interaction and restricted/repetitive behaviours) has also been reported in one person with the V59M mutation (7). No previous studies have systematically assessed the psychiatric morbidity in people with *KCNJ11* neonatal diabetes, or the impact that this has on families.

We aimed to characterise the types of psychiatric disorders present in children and adolescents with *KCNJ11* mutations, and explore the impact of these on families.

METHODS

Participants

We recruited 10 children from the UK (median age 8.5, range 6-17 years) with neonatal diabetes due to a mutation in the *KCNJ11* gene.

Study Procedures

Ethical approval

Ethical approval was obtained for the study from the National Research Ethics Service Committee South West - Exeter.

Recruitment and Consent

Patients were recruited at a neonatal diabetes family event in Exeter. Valid informed consent was obtained from parents (at the family event) and teachers (at a later date).

Developmental and physical health history

Parents reported the ages at which their child achieved major fine and gross motor, social and speech and language milestones, their child's educational

attainment, and any interventions required e.g. speech and language therapy, extra support at school. They also gave details of their child's physical and mental health history and current diabetes medication.

Psychiatric evaluation

Parents and teachers completed the DAWBA and SDQ. The DAWBA is a standardised diagnostic interview that combines structured and semi-structured approaches to generate DSM-IV (8) psychiatric diagnoses on 5-17-year-olds. Parallel versions exist for parents and young people aged 11-17 years, with a briefer questionnaire for teachers. It covers common emotional, behavioural and hyperactivity disorders as well as less common but sometimes more severe psychiatric disorders. Clinicians assess the data from all available informants to assign diagnoses according to DSM-IV. The initial DAWBA validation study showed excellent discrimination between community and clinic samples in rates of diagnosed disorder (9). Since then the DAWBA has been widely used in British national surveys and as a tool to aid clinical assessment in many countries.

The SDQ consists of 25 items relating to emotional symptoms, conduct problems, hyperactivity/ inattention, peer relationships and prosocial behaviour. Scores across the first four subscales (5 items each) are summed to create a 'total difficulties score' that ranges from 0-40. The impact supplement measures distress and social impairment caused by the child's difficulties. The reliability and validity of the SDQ make it a useful screen for psychopathology in children (10).

Data Analysis

The total difficulties, impact scores and psychiatric diagnoses were compared with normative data for approximately 8000 school-age children from the British Child and Adolescent Mental Health Survey 2004 (11).

RESULTS

Clinical and Developmental History

Parents reported high levels of developmental delay and learning difficulties (Table S1). 9/10 children required intervention to assist education or development. Psychiatric morbidity (Table S1) was recognised: 2 children had a clinical diagnosis of autism, one was awaiting assessment due to probable autism, and one had ‘autistic tendencies’. Another child required psychological support for low mood.

Strengths and Difficulties Questionnaire

Both parents (Figure 1) and teachers (Figure S1) reported high levels of psychopathology. Parent-reported median impact and median total difficulties Z-scores were 2.8 and 1.8 compared to the general school-age population (Z-score >1.3 psychiatric evaluation suggested). Problems were most marked in emotional difficulties and hyperactivity. Prosocial behaviour scores were also reduced compared to the background population (median Z-scores -1 and -0.7 for parent and teacher report respectively).

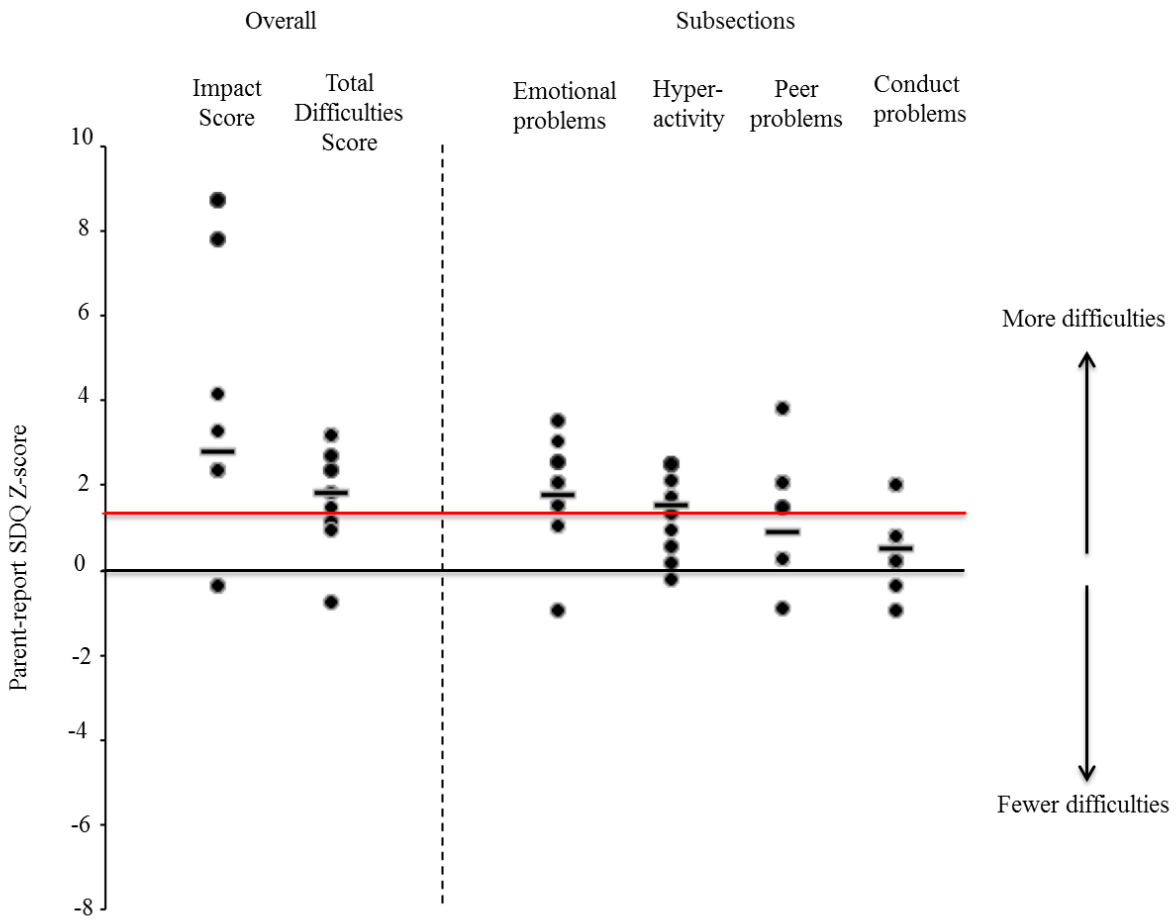

Figure 1. Patient difficulties as shown by parent-report SDQ scores (presented as Z-scores). Individuals are represented black circles and group medians as black bold horizontal lines. Zero on the x-axis represents school-age population mean, red horizontal line represents suggested clinical cut-point (90th percentile).

Development And Wellbeing Assessment (DAWBA)

Clinical evaluation of parents' and teachers' responses to the DAWBA questionnaire showed definite psychiatric disorder(s) were present in 6/10 children, but only 2 had a clinical diagnosis, with a further one awaiting formal assessment (Table 1). All children with either V59M (n=4) or R201C (n=2) had a definitive psychiatric diagnosis on the DAWBA. There was more than one disorder in 4/6 which was not recognised clinically. The prevalence of psychiatric disorder in British school children using the DAWBA is 10% (11), so the level of psychiatric morbidity is this cohort is higher than the background population (p=0.0001 for a one-sample proportion test).

Table 1. Clinical diagnoses and diagnoses obtained from DAWBA questionnaires

Case	Mutation	Clinical diagnoses	DAWBA diagnoses (DSM-IV classification)		
			Neurodevelopmental disorders	Anxiety disorders	Behavioural disorders
1	V59M	-	Autism ADHD[a] (combined)[b]	Other anxiety disorder	-
2	V59M	-	ADHD (combined)	-	-
3	V59M	Autism	Autism ADHD (combined)	Other anxiety disorder	-
4	V59M	Autism	Autism ADHD (combined)	Separation anxiety Specific phobia	-
5	R201C	-	-	Other anxiety disorder	-
6	R201C	-	Autism (probable)	Separation anxiety	Oppositional defiant disorder
7	K170R	-	-	-	
8	I182V	-	-	-	Other disruptive disorder (probable)
9	K170N	-	-	-	-
10	R201H	-	-	-	-

Table 1. Clinical diagnoses and diagnoses obtained from DAWBA questionnaires. [a] Attention Deficit Hyperactivity Disorder [b] (combined) denotes all three features (hyperactivity, impulsivity, inattention) present

Neurodevelopmental disorders were prominent (autism and ADHD). 3/10 children had both autism and ADHD; although DSM-IV criteria exclude ADHD as a diagnosis in the presence of autism, clinical practice and DSM-V has moved towards assigning both due to the high impact on families and need for

clinical intervention. Anxiety disorders were common with 5/10 children being diagnosed with at least one anxiety disorder.

Three children had additional probable diagnoses, but we could not make definitive diagnoses based on the DAWBA. Case 6 (R201C) was assigned a probable diagnosis of autism (and is awaiting formal diagnostic assessment for autism by his local services), and Case 8 (I182V) had a probable diagnosis of other disruptive (conduct) disorder, but this related to behaviour more than 6 months previously which was now resolving. Finally, Case 9 (K170N) was supported by health psychology and a nurture group for low mood and self-esteem, but did not reach the DAWBA diagnostic threshold for an emotional disorder.

DISCUSSION

Neurodevelopmental disorders (autism and ADHD) and/or anxiety disorders were present in all 6 children with sulphonylurea-treated neonatal diabetes due to V59M or R201C *KCNJ11* mutations. Most of these psychiatric disorders had not been diagnosed in clinical practice.

Definitive psychiatric diagnoses occurring only in those with the V59M or R201C mutation is consistent with the previous literature. These are the two commonest mutations associated with neurological/developmental features, which are almost invariable in V59M and inconsistently reported in R201C (3). There is a clustering in the type of psychiatric disorder; ADHD is present in all 4 children with the V59M mutation, consistent with previous reports of ADHD in

people and hyperactivity, inattention and impulsivity in mice with the mutation (2, 5, 6). Autism found in 4 children has previously been reported in a single patient (7). The presence of anxiety disorders in 4 children differs to the reduced anxiety behaviour noted in the V59M mouse model (6).

One of the most striking features of the assessment process was the impact on families of the difficulties identified. In those most severely impaired, parents had become full-time carers for their children. Some families reported that their children needed more support, which suggests that awareness of the psychological problems faced by such families should be raised amongst healthcare professionals involved in their care. The complex pattern of needs that we identified requires a fully integrated and collaborative approach involving parents, carers, GPs, paediatric endocrinologists, occupational therapists, clinical / educational psychologists, teachers, special educational needs coordinators and child and adolescent mental health services.

Limitations of the study

Due to the rarity of the condition, the number of participants recruited was small. In addition, the families who attended our family day may not be representative of all people with *KCNJ11* neonatal diabetes. Psychiatric difficulties could make patients more reluctant to attend a public meeting with considerable travelling or make them more likely to attend to seek advice. The total number of UK patients aged 5-17 with *KCNJ11* mutations at the time of the study was 21, therefore 48% of those eligible for inclusion in the study took part. Furthermore, our cohort did have significantly more V59M and R201C mutations than in the total UK paediatric cohort (60% v 26% p=0.049). For these reasons, we have

been unable to use this study to provide prevalence data on the psychiatric features associated with *KCNJ11* mutations. However our systematic and detailed assessments found considerable unrecognised psychiatric morbidity in this group.

Further work

Our research suggests that psychiatric morbidity predominantly affects people with V59M and R201C mutations and most of these mutation carriers are affected. A larger study assessing more patients with these and other mutations will give information on prevalence and the extent to which psychiatric morbidity forms part of a specific phenotype/genotype relationship. In addition, further studies are needed to assess the effects of sulphonylurea therapy on psychiatric symptoms in people with *KCNJ11* mutations.

Conclusions/Implications

Systematic assessment using standardised validated questionnaires reveals a range of psychiatric morbidity in children with *KCNJ11* neonatal diabetes. This is under-recognised clinically and has a significant impact on affected children and their families. An integrated and collaborative approach to clinical care is needed to ensure early identification and appropriate management of the complex needs of people with diabetes due to *KCNJ11* mutations.

FUNDING

ATH is supported by a Wellcome Trust Senior Investigator award (Grant number 098395/Z/12/Z). SEF has a Sir Henry Dale Fellowship jointly funded by the Wellcome Trust and the Royal Society (Grant Number 105636/Z/14/Z). MHS and BAK are supported by the NIHR Exeter Clinical Research Facility.

CONFLICTS OF INTEREST DISCLOSURES

None to declare.

ACKNOWLEDGEMENTS

Our thanks to the families who participated in the study.

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Supplementary material

Diabetic Medicine 2017 33(10):1387-91

Table S1. Patient characteristics and clinical information

Case	Age (yrs)	Sex	Mutation (Gene)	Parental Report of Developmental Milestones (Interventions)	Psychiatric history / epilepsy / other relevant clinical history	Current diabetes treatment (dosage in mg/kg/day[a])	HbA1c [b] IFCC mmols/mol (DCCT %)	Age at diagnosis of diabetes (weeks)	Age of transfer to sulphonylureas (if applicable)
1	14	M	V59M (*KCNJ11*)	Delayed – current mental age 4 (attends Special School)	'Some autistic tendencies' noted as a younger child. Epilepsy diagnosed aged 10 years; treatment Epilim (sodium valproate) 400mg BD.	Glibenclamide 10mg TDS (0.7mg/kg/day)	36 (5.4)	1	4 years
2	6	M	V59M (*KCNJ11*)	Delayed – current mental age 3 (1:1 support at school, SEN[d] statement)	Younger brother of case 3.	Glibenclamide 10mg BD (1mg/kg/day)	30 (4.9)	9	On SU since diagnosis
3	17	M	V59M (*KCNJ11*)	Delayed – current mental age 3 (attends Special School)	Autism diagnosis. Under local [g]CAMHS team.	Glibenclamide 30mg breakfast, 35mg dinner (1mg/kg/day)	33 (5.2)	1	10 years
4	6	F	V59M (*KCNJ11*)	Delayed – current mental age 3 (25 hours/week 1:1 support at school, SEN statement, uses Makaton to aid communication)	Autism diagnosis July 2014. Speech improved following recent increase in SU from 2.6mg / day.	Glibenclamide 11mg BD (1mg/kg/day)	38 (5.6)	12	12 months
5	16	F	R201C (*KCNJ11*)	Developmental concerns re: social interaction, learning difficulties at school (on enhanced learning programme – current mental age 12)	Difficulties with language expression / comprehension and mathematics.	Glibenclamide 20mg breakfast, 15mg lunch, 20mg dinner (1.1mg/kg/day)	55 (7.2)	11	10 years
6	9	M	R201C (*KCNJ11*)	Speech delay (speech and language therapy when younger, support at school from teaching assistant)	Probable autism – awaiting formal assessment.	Glibenclamide 4.2mg breakfast, 3mg lunch, 3.7mg dinner (0.3mg/kg/day)	37 (5.5)	1	4.5 months
7	8	M	K170R (*KCNJ11*)	Speech delay (speech & language therapy, 20 hours/week support from SENCO[f], IEP[e])	Being assessed for dyslexia (difficulties with maths/ literacy/ spellings /letter and shape formation).	Glibenclamide 5mg breakfast, 5mg lunch, 2.5mg dinner (0.48mg/kg/day)	32 (5.1)	33	15 months
8	15	F	I182V (*KCNJ11*)	Mild speech delay (some 1:1 support at school, particularly during examinations)	Diagnosed with 'borderline' dylsexia. Spent some time on gliclazide; found concentration improved when switched to glibenclamide.	Glibenclamide 15mg breakfast, 10mg dinner; noted to not be taking her medication regularly (0.5mg/kg/day)	127 (13.8)	1	N/A[c] (TNDM[h]); Insulin 8-9 months, no Rx then SU aged 11 years
9	8	M	K170N (*KCNJ11*)	Mild speech and motor delay (speech and language therapy as toddler)	Attends 'nurture group' at school to help confidence. Excels academically. Under paediatric diabetes psychology service due to low mood related to diabetes.	Glibenclamide 5mg breakfast, 2.5mg dinner (0.29mg/kg/day)	33 (5.2)	11	5 months
10	6	F	R201H (*KCNJ11*)	Normal	Nil	Glibenclamide 2.5mg TDS (0.4mg/kg/day)	42 (6.0)	12	12 months

[a]Where no recent weight available, weight calculated based upon CDC paediatric growth charts using 50th percentile (http://www.cdc.gov/growthcharts 2000) [b] Most recently available result [c]N/A = Not applicable [d]SEN = special educational needs [e]IEP = Individualised education program [f]SENCO = special educational needs coordinator [g]CAMHS = child and adolescent mental health services [h]TNDM = Transient neonatal diabetes mellitus

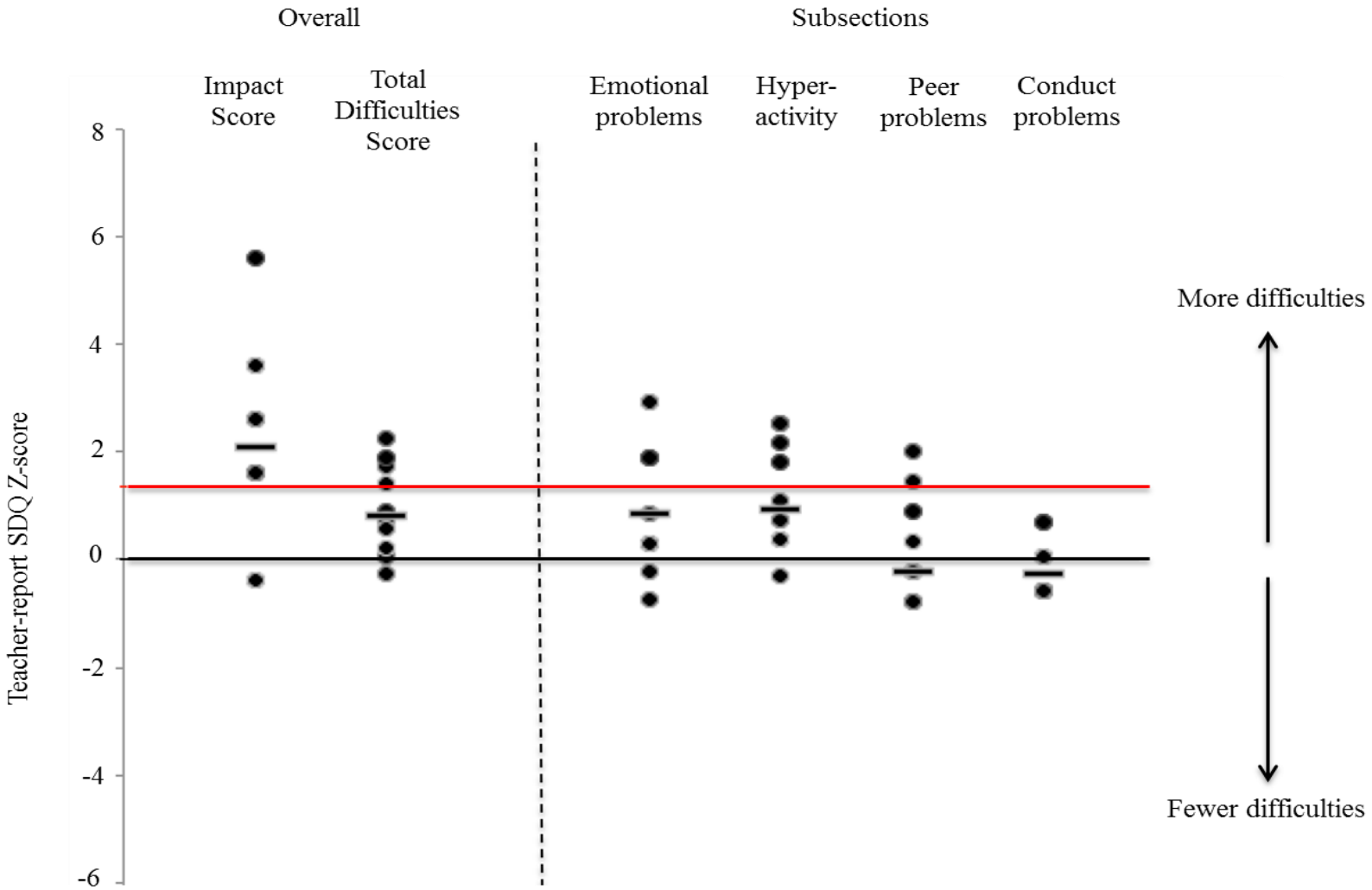

Figure S1. Patient difficulties as shown by teacher-report SDQ scores (presented as Z-scores). Individuals are represented as black circles and group medians as black bold horizontal lines. Zero on the x-axis represents school-age population mean, red horizontal line represents suggested clinical cut-point (90th percentile).

CHAPTER 3:

PART B

Neuropsychological impairments in children with *KCNJ11* neonatal diabetes

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ACKNOWLEDGEMENTS / CONTRIBUTIONS

ATH, PB, TJF and JT contributed to conception and design of the study. EB, LP, MR, EB, TJF, BK, MS, and JT contributed to data acquisition. JT, LP and MR performed neuropsychological testing with support from members of the Exeter clinical research team. JT, EB, TJF, ATH, SEF, and MS contributed to analysis and interpretation of data. All other authors critically revised the article and approved the final version prior to submission.

MY CONTRIBUTIONS TO THE CHAPTER

I designed the study and developed the protocol with ATH, TJF, JT and BK. I recruited participants and their parents and collected clinical data including developmental histories with support from BK and MS. I performed the data analyses with EB and interpreted the findings with support from TJF, ATH, SEF, MS and EB. I provided feedback on the study findings to participants with support from JT and TJF. I produced the main figure. I wrote the manuscript and revised this according to feedback from co-authors.

We support the findings of Carmody et al who offered new insights into the neurological phenotype of people with *KCNJ11* neonatal diabetes[1]. Neurological features result from the K-ATP channel affected by these mutations being expressed in the brain as well as the pancreas [2]. Past work has characterised developmental delay associated with specific mutations e.g. V59M, known as DEND syndrome (developmental delay, epilepsy, and neonatal diabetes) [3, 4]. Affected individuals also have impaired visuomotor performance [5] and psychiatric (predominantly neurodevelopmental) disorders [6]. Carmody et al described neuropsychological impairments in children with *KCNJ11* mutations and compared their cognitive functioning with unaffected sibling controls, concluding that affected children without global developmental delay had lower IQs and performed worse on a range of assessments of academic achievement and executive function than their unaffected siblings [1].

Characterising the neuropsychological profiles in this rare form of diabetes is important to enable appropriate educational support to be implemented early for affected individuals. Here we report the results of neuropsychological testing in 10 individuals with *KCNJ11* neonatal diabetes who received a molecular genetic diagnosis in Exeter, UK.

Ethical approval for the study was obtained from the Devon and Torbay Research Ethics Committee.

We recruited 10 children (median age 8.8 years (range 5.9-17.0 years)) with neonatal diabetes due to a mutation in the *KCNJ11* gene (4 V59M, 2 R201C, K170R, K170N, R201H, I182V), and 7 unaffected siblings (median age 11 years, range 4-15 years). All children with neonatal diabetes were sulphonylurea-treated at the time of testing (median dose 0.56 mg/kg/day, range 0.09-1.15mg/kg/day). Nine children had permanent neonatal diabetes

(PNDM) and one (with the I182V mutation) had transient neonatal diabetes (TNDM) which had relapsed 4 years prior to taking part in this study. Informed consent was obtained from parents. Parents were asked about their child's developmental milestones, educational attainment, and any interventions at school. Children were assessed by clinicians in the research team using 8 tests selected from specific batteries to measure a wide range of functioning. The 'narrative memory' subscale of the Developmental Neuropsychological Assessment (NEPSY-II) was used to test episodic memory, and the 'verbal fluency' subtest of the Delis-Kaplan Executive Function System (DKEFS) was used to test letter fluency. The 'symbol search', 'digit span', and 'vocabulary', subtests of the Wechsler Intelligence Scale for Children, fourth edition (WISC-IV) were used to test processing speed, memory capacity/working memory, and verbal comprehension. Finally, all three subtests of the Beery-Buktenica Test of Visual Motor Integration (VMI), ('visual perception', 'motor coordination' and 'visual motor integration' tests), were used to assess fine motor skills and hand-eye coordination. On completion the tests were marked by a Paediatric Clinical Neuropsychologist and scores were converted to Z-scores using normative data for the school age population.

All four children with the V59M mutation had severe developmental delay and 2 had a clinical diagnosis of autism; 2 attended a special school and 2 had statements of special educational needs with one to one support required at school. Two were untestable using our neuropsychological battery and 2 scored the lowest of all participants in the few tests they did complete (Z-scores ≤ -3). The remaining 6 children did not have mutations consistently associated with severe developmental delay, however parents reported speech delay or

learning problems requiring support at school in 5 individuals. Specifically, 3 children had required speech and language therapy (R201C, K170R, K170N), and 4 children required support at school in the form of enhanced / individualised learning programmes and / or one to one support from a teaching assistant or special educational needs coordinator (2 R201C, K170R, I182V). Median Z-scores in these 6 children were below school-age population average in all tests (figure 1), and were particularly low (>1SD below population average) in tests of executive function (verbal fluency), verbal comprehension (vocabulary) and visuo-motor performance (VMI). Only 1 child (K170N) scored within the average range in all tests completed. In 7 sibling controls, median scores were within the normal range in all tests.

Our findings using a different battery of tests are consistent with the study by Carmody, where only 2/9 individuals with global developmental delay were able to complete any neuropsychological tests and scores in tests they did complete were the lowest of the group. In those individuals without global developmental delay, compared to unaffected sibling controls, Carmody reported lower scores in the WISC-IV digit span test (working memory); these were also evident in our assessments. Carmody's study showed impairments in vocabulary assessed using the WASI-II; similarly, we found impairments in the vocabulary subtest of the WISC. The low scores we observed in the various components of the VMI were consistent with impaired visuo-motor performance in people with *KCNJ11* mutations reported by the same group [5].

Other published studies also suggest difficulties are not restricted to individuals with known DEND mutations. Landmeier reported disturbances in parent-reported social-emotional and regulatory behaviours, attention, sleep and learning that occurred in all *KCNJ11* mutation subtypes studied [7]. Furthermore, Busiah found attention deficits in all patients and dyspraxia in 81% of people with *KCNJ11* mutations not previously known to have neurological sequelae [8].

Due to the rarity of the disease the numbers in our study are small, but nevertheless this work supports Carmody et al and provides further evidence of neuropsychological dysfunction in people with *KCNJ11* mutations, not limited to those with known DEND mutations. We advocate early neuropsychological assessment as part of the multidisciplinary care of these individuals to facilitate provision of targeted educational support. Further research in this area is needed to assess the impact of high dose sulphonylurea and timing of initiation of sulphonylurea on neurodevelopment in *KCNJ11* neonatal diabetes.

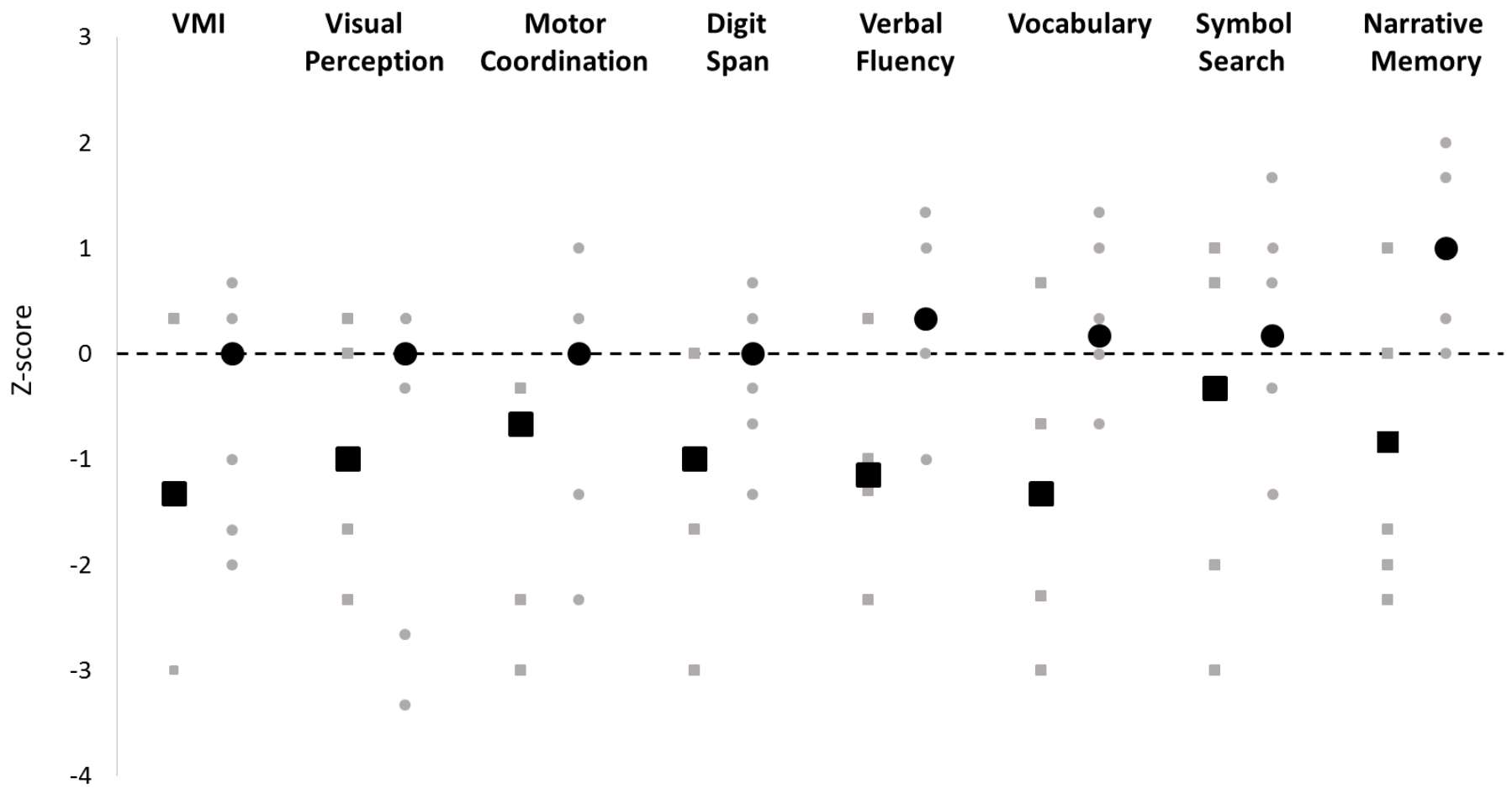

Figure 1. Neuropsychological test scores represented as Z-scores. X-axis represents school age population mean. Large black squares are *KCNJ11* group medians, large black circles are sibling control group medians. Small grey squares / circles represent individual scores for *KCNJ11* participants (n=3-6) and sibling controls (n=5-7).

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CHAPTER 4

Cognitive, neurological and behavioral features in adults with *KCNJ11* neonatal diabetes

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AZ, LT, AC, ATH, and BAK contributed to conception and design of the study. AZ, LT, JD, ATH, MHS and SEF contributed to data acquisition. AZ and LT performed the history-taking, neurological examinations and neuropsychological testing. PB, JD, AZ, LT, TJF, ATH, SEF, and MHS contributed to analysis and interpretation of data. All other authors critically revised it. All authors approved the final version prior to submission.

MY CONTRIBUTIONS TO THE CHAPTER

I contributed to the development of the study protocol and collection of clinical data. I analysed the data with JD. I interpreted the study findings with support from AZ, LT, JD and ATH. I produced the figures and tables with support from JD. I wrote the manuscript and revised it according to feedback from co-authors prior to submission.

Abstract

Objective

Central nervous system (CNS) features in children with permanent neonatal diabetes (PNDM) due to *KCNJ11* mutations have a major impact on affected families. Sulfonylurea therapy achieves outstanding metabolic control, but only partial improvement in CNS features. The effects of *KCNJ11* mutations on the adult brain and their functional impact are not well described. We aimed to characterise the CNS features in adults with *KCNJ11* PNDM, compared to adults with *INS* PNDM.

Research Design and Methods

Adults with PNDM due to *KCNJ11* mutations (n=8) or *INS* mutations (n=4) underwent a neurological examination, and completed standardised neuropsychological tests/questionnaires about development/behavior. Four individuals in each group underwent a brain MRI scan. Test scores were converted to Z-scores using normative data, and outcomes compared between groups.

Results

In individuals with *KCNJ11* mutations, neurological examination was abnormal in 7/8; predominant features were subtle deficits in coordination/motor sequencing. All had delayed developmental milestones and/or required learning support/special schooling. Half had features and/or a clinical diagnosis of autism spectrum disorder. *KCNJ11* mutations were also associated with impaired attention, working memory and perceptual reasoning, and reduced IQ (median

IQ *KCNJ11* vs *INS* mutations 76 vs 111, p=0.02). However, no structural brain abnormalities were noted on MRI. The severity of these features was related to the specific mutation and they were absent in individuals with *INS* mutations.

Conclusions

KCNJ11 PNDM is associated with specific CNS features which are not due to long-standing diabetes, persist into adulthood despite sulfonylurea therapy, and represent the major burden from *KCNJ11* mutations.

Introduction

KCNJ11 gene mutations are the commonest cause of permanent neonatal diabetes (PNDM), which presents in the first 6 months of life and affects 1 in 100,000 live births (1). *KCNJ11* is expressed in the pancreas and brain as well as other tissues, and encodes the Kir6.2 subunit of the ATP-dependent potassium (K_{ATP}) channel. In the pancreas, the K_{ATP} channel links increasing blood glucose to insulin secretion, but activating *KCNJ11* mutations prevent channel closure in response to metabolically generated ATP and result in diabetes (2). Clinically, patients present in an insulin-deficient state and prior to discovery of disease-causing variants in the *KCNJ11* gene they required insulin therapy. It was later shown that *KCNJ11* PNDM could be treated with sulfonylurea tablets, which bind and close the channel allowing insulin secretion, excellent metabolic control and reduced glycaemic variability (3). For many patients and their families transferring from insulin to oral sulfonylureas vastly improved quality of life in relation to their diabetes (4).

Central nervous system (CNS) features occur in children with *KCNJ11* PNDM in addition to diabetes. These are thought to result from expression of aberrant K_{ATP} channels in the brain. The precise role(s) of K_{ATP} channels in the human CNS has not been fully elucidated, but rodent studies suggest that they play a role in glucose sensing and homeostasis as well as seizure propagation (5; 6). *KCNJ11* is expressed in many brain areas but there are particularly high levels of expression in the cerebellum (7; 8). The cerebellum is well known for its role in motor learning and coordination (9), but it also has functions relating to language, executive function and to mood; furthermore, cerebellar abnormalities have been linked with autism (10; 11). Documented CNS features in children with *KCNJ11* mutations range from subtle neuropsychological impairments that specifically affect attention, praxis and executive function to the severe and overt DEND/intermediate DEND syndrome (developmental delay, epilepsy and neonatal diabetes) (12-15). Other associated features may include psychiatric morbidity, specifically neurodevelopmental disorders and anxiety disorders, visuomotor impairments, and sleep disturbance (16-18). The severity of the CNS phenotype is related to the genotype. For example, the V59M mutation is frequently associated with iDEND syndrome and neurodevelopmental features whereas the R201H mutation, previously associated with diabetes alone, has been more recently linked with subtle neuropsychological features (12). Historically, the severity of CNS features was thought to be related to the functional severity of the specific mutation *in vitro*, although functional interpretation also has to take into account the impact of the mutation on the open probability of the K_{ATP} channel which will depend on whether it affects channel gating or ATP binding (19-22).

Sulfonylurea treatment results in partial improvement in the CNS features (23-26), and resolution of functional cerebellar and temporal lobe abnormalities on single-photon emission computed tomography (SPECT) scanning (24; 27). The improvement in CNS features may be limited as a result of poor penetration of the sulfonylurea across the blood brain barrier or active transport back out of the brain, leading to sub-therapeutic concentrations in the cerebrospinal fluid (CSF) (28). This, and anecdotal clinical experience of greater CNS response with higher doses of sulfonylurea, has prompted clinical recommendations of glyburide doses of ~1mg/kg/day in people with severe neurological features secondary to *KCNJ11* mutations (29). However, the neurobehavioral features continue to have a huge impact on families despite sulfonylurea treatment (16). This contrasts markedly with the outstanding metabolic response that changed lives by alleviating the anxiety associated with poor metabolic control (4). A key question is whether the CNS features continue to represent the major burden from *KCNJ11* mutations in adult life. To date all studies characterising CNS features in *KCNJ11* PNDM have been conducted in predominantly paediatric cohorts (12-14; 16; 23). However, brain development continues beyond childhood and adolescence (30; 31). No study has comprehensively assessed the CNS outcomes in adults with *KCNJ11* mutations.

Mutations in the *INS* gene are a less common cause of neonatal diabetes, accounting for around 10% of cases (1). Heterozygous dominant negative *INS* mutations often affect protein synthesis resulting in production of structurally abnormal preproinsulin and proinsulin within the beta cell, endoplasmic reticulum (ER) stress and cell death. Individuals with these mutations also typically present with insulin deficiency, but unlike *KCNJ11* PNDM, require lifelong treatment with

replacement doses of insulin (32). The *INS* gene is not expressed in any significant levels in the brain, therefore it is very unlikely that individuals with *INS* mutations would display a characteristic CNS phenotype as a direct result of their mutations (33). In fact, there have not been any reports of any such neurological issues, in contrast to those with *KCNJ11* PNDM.

Individuals with PNDM may have long-term CNS sequelae secondary to diabetic ketoacidosis at diagnosis, as seen in Type 1 diabetes (34; 35). However, cerebral oedema in *KCNJ11* PNDM gives rise to a pattern of neurological impairment distinct from that seen as a direct result of brain K_{ATP} channel dysfunction (36). More subtle neurocognitive problems also occur in the presence of diabetes per se, particularly if metabolic control is poor and diabetes is diagnosed before age 7 (37). Further, individuals with Type 2 diabetes are at increased risk of developing Alzheimer's disease in later life, and this may be in part due to chronic metabolic disturbance and changes in insulin signalling (38). Indeed, there is evidence from both animal and human studies that insulin plays a key role in central processes including memory and learning (38). The non-specific diabetes-related cognitive features could confound assessment of CNS phenotype in people with *KCNJ11* mutations, however people with *INS* mutations are well placed to control for them. There has been no previous detailed comparison of the CNS phenotype in people with *INS* and *KCNJ11* mutations.

Aims

The aims of the study were to characterise the neurological and neuropsychological features in adults with *KCNJ11* PNDM, and to compare these with adults with *INS* PNDM.

Methods

Ethical approval

Ethical approval was obtained from the National Research Ethics Service Committee South West-Exeter.

Sample size and patient recruitment

We identified 34 patients >16 years old with *KCNJ11* mutations and 9 patients >16 years old with *INS* mutations who had received a molecular genetic diagnosis in Exeter and who had been diagnosed with permanent neonatal diabetes under 6 months of age. We approached potential participants either directly at a neonatal diabetes family event in Exeter or via the Consultants in charge of their clinical care. We invited 17 individuals with *KCNJ11* mutations to join the study; of these, 10 agreed to participate. However, 2 individuals were excluded from the analysis due to possible confounding factors: one individual (mutation L164P) was excluded because he was taking antipsychotic medication to treat a psychotic illness at the time of the study and had had a particularly severe initial presentation with diabetic ketoacidosis and 3 days in a coma, and a second individual (mutation V59M) was excluded due to severe neurological impairment following initial presentation with diabetic ketoacidosis (further clinical

characteristics of excluded participants are available in online supplemental Table S1). We approached 9 individuals with *INS* mutations and 4 agreed to take part. All participants were from the UK apart from one who was from Canada.

Tests

All participants were visited at home or assessed in the Exeter Clinical Research Facility by the same Consultant neurologist and Consultant clinical neuropsychologist who carried out the history taking using a standard proforma, neurological examination, neuropsychological assessments, mood questionnaire and neurodevelopmental screen. If possible an informant or carer was also present to facilitate information gathering. The severity of intellectual impairment and behavioral disturbance in one individual (*KCNJ11-8* [V59M]) meant that it was not possible for him to attempt any of the cognitive tests. Another individual (*KCNJ11-6* [V252G]) did not wish to attempt the Controlled Oral Word Association Test (COWAT) and was unable to understand instructions for the Colour Trails Test (CTT). In 8 participants (4 with *KCNJ11* mutations and 4 with *INS* mutations), T2-weighted brain magnetic resonance imaging (MRI) scans were performed using a 1.5T MRI scanner. The scans were reviewed and interpreted by a radiologist and a neurologist who were blinded to the mutation status of the individuals concerned.

Medical / developmental history, educational and professional attainment

Participants and informants were asked for a standard medical history, the ages at which major milestones were attained, whether learning support was required and level of education / employment.

Neurological Examination

A full neurological examination was performed. This included assessment of cranial nerves, limb tone, power, reflexes, coordination, sensation, and simple tests of motor sequencing and praxis, comprising 2 tests of bimanual coordination, one unilateral motor sequencing task (the Luria three hand position test), copying unfamiliar hand positions and manual miming, both tested in each hand.

Psychiatric and neurodevelopmental screen

Current psychological distress was assessed using the Hospital Anxiety and Depression Scale (HADS) questionnaire. The Autism Spectrum Quotient (AQ) was administered to screen for autistic traits.

Cognitive function

A battery of neuropsychological tests were administered to assess a variety of cognitive domains. The Wechsler Abbreviated Scale of Intelligence (WASI) was used as a brief measure of current IQ. The Verbal Paired Associates and Visual Reproduction subtests of the Wechsler Memory Scale (WMS-IV) were used to give a verbal and non-verbal (visual) measure of memory. Subtests of the Wechsler Adult Intelligence Scale, 4th edition (WAIS-IV) were administered: cancellation to assess processing speed and digit span (forwards and backwards) to assess working memory. Subtests of the Visual Object and Space Perception battery (VOSP) assessed visuospatial function; incomplete letters and object decision to test object perception, and dot counting and cube analysis to

test spatial perception. The Controlled Oral Word Association Test (COWAT) was used to assess aspects of executive function including verbal fluency, self-monitoring and ability to assimilate and adhere to stipulated rules. The Colour Trails Test (CTT) 1 and 2 were used as measures of sustained and divided attention, hand eye motor coordination and speed. Finally, the Addenbrooke's Cognitive Examination-Revised (ACE-R) was used as a broad screening measure of cognition, providing an assessment of the following cognitive domains; attention/orientation, memory, fluency, language, and visuospatial function.

Functional assessment

The Cambridge Behavioral Inventory Revised (CBI-R) questionnaire was used to complement the information obtained from the history taking. This measure seeks the opinion of the informant e.g. carer or family member on the frequency of a range of behaviors in the domains of memory and orientation, everyday skills, self-care, abnormal behavior (e.g. tactlessness, impulsiveness), mood, unusual beliefs, altered eating habits, disturbed sleep, stereotypic and motor behaviors, and altered motivation. For each behavior the informant assigned a score of 0-4 based on the frequency: scores of 3 (occurring daily) or 4 (occurring constantly) denote a significant behavioral deficit.

Statistical analysis

Data were analysed using Excel 2010 and Stata 14. Qualitative data were presented descriptively. Where population normative data were available, neuropsychological test scores were converted to Z-scores. For VOSP subtests,

a pass was a score ≥5th population percentile. To compare characteristics and outcomes between the *KCNJ11* and *INS* groups, data were analysed using non-parametric methods (Mann-Whitney test for numerical variables and Fisher's exact test for categorical variables). Data are presented as median (range) unless otherwise stated.

Results

Participant Characteristics

Baseline clinical characteristics of the participants are outlined in Table 1; these were similar between individuals with *KCNJ11* mutations and individuals with *INS* mutations.

Neurological Features

Abnormalities on neurological examination were identified in 7/8 *KCNJ11* participants and only one *INS* participant (Table 2).

Table 1. Baseline characteristics in individual participants, *KCNJ11* cases and *INS* controls and group characteristics summary.

Case	Mutation	Inheritance	Sex	Age	Age at diabetes diagnosis (weeks)	Age at genetic diagnosis (years)	Age at transfer to SU (years)	Treatment (total daily dose)	HbA1c DCCT (%)	HbA1c IFCC (mmol/mol)
KCNJ11 1	G53S	Autosomal dominant	M	32	2	23	23	Glyburide 30mg, metformin 1g	9.3	78
KCNJ11 2	R201H	Presumed *de novo*	F	22	4	15	15 (attempted)	Insulin (restarted after trial of glyburide)	8.1	65
KCNJ11 3	R201H	*De novo*	M	36	10	29	29	Gliclazide (120mg)	8.1	65
KCNJ11 4	R201C	Presumed *de novo*	F	36	5	27	34	Glyburide (40mg)	7.0	53
KCNJ11 5	R201C	*De novo*	M	19	6	13	13	Glyburide 27.5mg	5.4	36
KCNJ11 6	V252G	*De novo*	M	28	8	21	21	Glyburide (85mg)	10.8	95
KCNJ11 7	V59M	*De novo*	F	25	15	17	17	Glyburide (7.5mg)	8.1	65
KCNJ11 8	V59M	*De novo*	M	17	5	10	11	Glyburide (55mg)	5.9	41
INS 1	C43F	Autosomal dominant	F	35	78	31	N/A	Insulin	NK	NK
INS 2	F48C	Presumed *de novo*	F	50	5	42	N/A	Insulin	NK	NK
INS 3	G75C	*De novo*	M	28	8	26	N/A	Insulin	7.9	63
INS 4	H29D	*De novo*	F	20	26	12	N/A	Insulin (pump)	8.2	66
KCNJ11 group	N/A	*De novo = 7 (87.5%) Autosomal dominant = 1 (12.5%)*	M = 5 (63%) F = 3 (37%)	26.5 (17-36)	5.5 (2-15)	19 (10-29)	21 (11-34)	Insulin treated = 1/8	8.1 (5.4-10.8)	65 (36-95)
INS group	N/A	*De novo = 3 (75%) Autosomal dominant = 1 (25%)*	M = 1 (25%) F = 3 (75%)	31.5 (20-50)	17 (5-78)	28.5 (12-42)	N/A	Insulin treated = 4/4	8.1 (7.9-8.2)	65 (63-66)
P value	N/A	1.0	0.55	0.44	0.15	0.23	N/A	0.01	0.79	0.79

Table 1. Summary numerical data are presented as median (range) and categorical variables are presented as n (%). Mutations were presumed to have arisen *de novo* if there was no parental history of diabetes but the mutation status of the parents had not been confirmed with a genetic test. HbA1c values are the results available closest to the time of the neurobehavioral assessment. N/A = not applicable, NK = not known.

Table 2 - History and examination findings for individual *KCNJ11* cases and *INS* controls.

Case (mutation)	HISTORY							EXAMINATION / INVESTIGATIONS	
	Developmental milestones / interventions	**Seizures (cause, if known)**	**Educational attainment**	**Learning difficulties / support**	**Employment status (job)**	**Employment status (job) of parents / siblings**	**Psychiatric history**	**Neurological examination**	**Brain MRI**
KCNJ11 1 (G53S)	D (speech and motor)	No	MS then SS (age 11)	LS (repeated year 2)	E (supermarket)	F – E (council), S – E (chemicals factory)	Repetitive hand washing and rigid routines.	Impaired motor sequencing, fine finger movements, praxis. Subtle nystagmus, dysdiadokokinesis.	ND
KCNJ11 2 (R201H)	D (speech)	No	MS then U (nursing and childcare)	LS (English until age 15, Maths for 1 year)	US	F – E (accountant) M – E (nurse)	Nil	Intermittent mild head titubation. Brisk reflexes, questionable increase in tone in left arm.	N
KCNJ11 3 (R201H)	N	Yes – hypoglycaemia on insulin	MS then C (18 months, NVQs levels 1 &2)	LS (English and Maths).	E (baker)	NK	Nil	Normal	Bilateral high T1 signal in pons – artefact
KCNJ11 4 (R201C)	N	Yes	MS then C (computing module)	LS (throughout school)	E (pubs, shops, hotel)	B – E (operations manager), S – E (sports complex manager)	Depression – Sertraline 50mg. Occasional auditory hallucinations.	Nystagmus (2-3 beats on horizontal gaze), subtle intention tremor.	N
KCNJ11 5 (R201C)	D (gross motor, speech).Speech therapy.	Yes – hypoglycaemia on insulin	MS then C (for people with ID)	LS (from age 10).	CS (college for people with ID).	F – E (company manager), M - UE (housewife), B – E (aviation engineer), B – US (political science)	Anxiety (social skills training by psychologist)	Impaired motor sequencing.	ND
KCNJ11 6 (V252G)	D (speech, fine motor). Speech therapy.	No	SS then school for autistic children	Unable to read/write. Supported accommodation.	UE	B – E (carpenter)	Autism	Impaired motor sequencing, praxis, heel-toe walking. Hand-flapping.	ND
KCNJ11 7 (V59M)	Speech therapy.	Yes – hypoglycaemia on insulin	MS then C (1st year - childcare)	LS (throughout school)	E (SS teaching assistant)	M – E (SS teaching assistant), F – E (lecturer), B – E (trainee lawyer)	Anxiety	Impaired motor sequencing, dysdiadokokinesis, choreiform movements.	N (movement artefact)
KCNJ11 8 (V59M)	D (global).	Yes – hypoglyca	SS	Unable to read/write.	UE	S – E (museum curator)	Autism	Ritualistic, clumsy, echolalia. Generally	ND

		emia on insulin						uncooperative with examination.	
INS 1 (C43F)	N	Yes	U (pharmacy degree)	No	E (hospital pharmacist)	NK	Nil	Normal	ND
INS 2 (F48C)	N	Yes – hypoglycaemia	U (law degree)	No support.	UE (ill health). Clerical / carer jobs in past	F – E (taxi business manager, security guard)	Depression (medication and CBT in the past), OCD	Depressed leg reflexes (in-keeping with known diabetic neuropathy).	Left temporal lobe abnormality – CSF space or artefact
INS 3 (G75C)	N	No	MS	No support.	E (dept. of work and pensions)	F – E (carpenter, transport manager), B – E (marketing agency)	Nil	N	N
INS 4 (H29D)	N	Yes – DKA	MS	LS (Maths & English 4 years).	E (bank call centre)	F – E (carpenter)	Anxiety, panic attacks (Escitalopram in the past)	N	Prominent left cerebellar sulcus, periventricular white matter lesions

Table 2. NK = not known, ND = not done, D=delayed, N=normal, MS = Mainstream school, SS = Special school, U = University, C = College, LS = learning support, E = employed, UE = unemployed, ID = intellectual disability, US = university student, CS = college student, OCD = obsessive compulsive disorder, DKA = diabetic ketoacidosis, F = father, M = mother, B = brother, S = sister

Developmental History, Educational and Professional Attainment

Developmental histories, level of educational support and employment history reported for each participant are described in Table 2. Developmental delay and / or learning difficulties were present in all *KCNJ11* participants and they continued to require high levels of support as adults. In contrast, the *INS* group did not report major learning difficulties in keeping with their subsequent employment history and independence in adulthood.

Neurodevelopmental and Psychiatric Features

4/8 with *KCNJ11* mutations, but none of the participants with *INS* mutations, had features of autistic spectrum disorder, either via a clinical diagnosis of autism or an AQ score at or above the threshold suggestive of clinically significant autistic traits (Table 2 and S3). Two individuals in the *KCNJ11* group and one in the *INS* group required treatment either at the time of the study or in the past for depression or anxiety. HADS scores for anxiety and depression were similar in *KCNJ11* vs *INS* participants (Table S3). One individual in the *KCNJ11* group and 2 individuals in the *INS* group scored above the HADS clinical threshold (11) for anxiety (Table 2, Table S3).

Cognitive Function

IQ was lower in the *KCNJ11* group vs the *INS* group (IQ 76(55-101), n=7 and 111(90-124), n=4, p=0.02). Three individuals in the *KCNJ11* group had IQs <70 (Table S2) and impairments in adaptive behaviors in-keeping with a clinical diagnosis of intellectual disability (39). 5/7 individuals in the *KCNJ11* group

scored below the clinical cut-point for cognitive impairment on the ACE-R (Figure S1). CTT1 scores suggested reduced attention (CTT1 Z-score -1.7(-3.0- -0.1), n=6 vs 0.4(-1.1-1.2), n=4, p=0.03, CTT2 Z-score -0.8(-3.0-0.8), n=6 vs. 0.7(-1.0-1.2), n=4, p=0.13 (Figure 2, Table S2)).

In the *KCNJ11* group but not the *INS* group median scores in the WASI, WAIS and WMS were below population average in all subtests apart from the verbal paired associates subtest of the WMS (Figure 1). Scores were particularly low (≤2SD below population average) in the matrix reasoning component of the WASI (Z-score -3.2(-4.8- -0.9) vs. 0.6(-0.7-0.8), p=0.008) and the digit span component of the WAIS-IV (Z-score -2.0(-3.0-0.3) vs 0(-1.0-0.3), p=0.046). Cancellation scores, although not as markedly reduced compared to population norms, were significantly lower in the *KCNJ11* group (Z-score -1(-3-0) vs 2.8(0.7-3.0), p=0.007). COWAT and VOSP scores showed a trend towards reduced executive function and visuospatial function respectively in the *KCNJ11* group (Table S3), although these did not reach statistical significance.

Behavioral / functional impact

In the *KCNJ11* group, 6 individuals had severe behavioral features which clustered in the domains of everyday skills (5/6), stereotypic behavior (5/6), memory and orientation (4/6), abnormal behavior (3/6), mood (3/6), and motivation (3/6). Specific everyday skills highlighted included writing (3/5) and dealing with money/bills (2/5). The most frequent stereotypic behaviors were being rigid / fixed (3/5) and having fixed routines (4/5). Poor concentration was

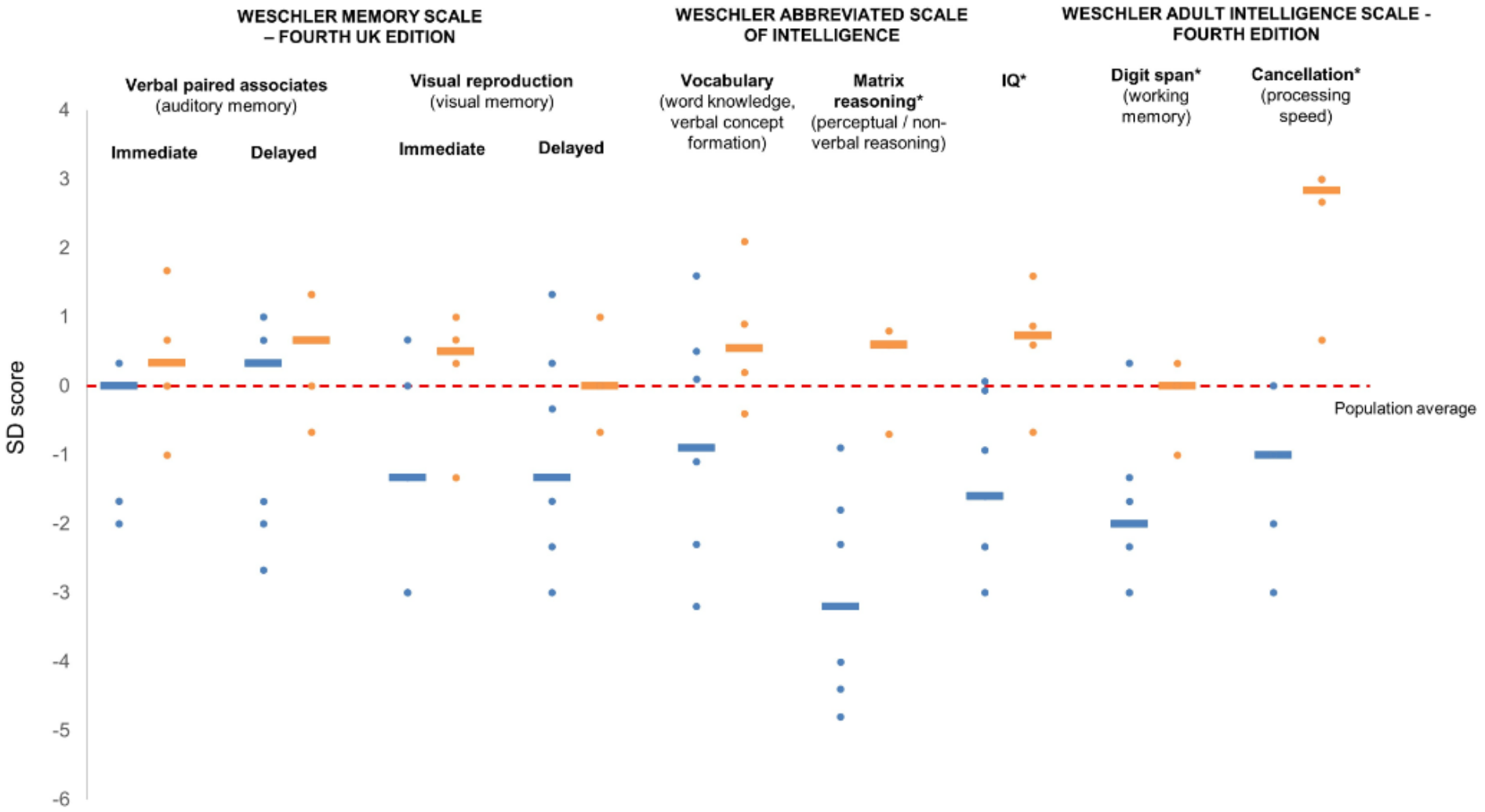

Figure 1: Neuropsychological testing in *KCNJ11* patients (blue, n=7) and *INS* patients (orange, n=4). WMS: Verbal paired associates I/II (immediate/delayed) – auditory memory, Visual reproduction I/II (immediate/delayed) – visual memory (both components of working memory) WASI: Vocabulary - word knowledge and verbal concept formation, matrix reasoning – non-verbal/perceptual reasoning. Digit span – working memory, cancellation – processing speed. *denotes $p<0.05$ for difference between *KCNJ11* and *INS* groups.

highlighted as a specific feature in all 4 individuals who had memory and orientation problems. Two individuals had significant difficulties with self-care and 2 reported disturbed sleep.

Neuroimaging

Structural brain MRI was normal in participants with *KCNJ11* mutations. In *INS* controls, 2 were normal, and 2 had minor abnormalities which were not clinically significant (Table 2).

Severity of impairments associated with the specific mutation

Performance in the cognitive tests was better in the 2 individuals with the R201H mutation. These individuals consistently had scores equal to or greater than the *KCNJ11* group medians (Figures 1 and 2, Table S2), and no significant behavioral features were reported.

Discussion

We have characterised for the first time the profile of neurological, neuropsychological and behavioral features present in adults with PNDM due to *KCNJ11* mutations. The key features were learning difficulties, features of ASD, subtle motor deficits affecting coordination and motor sequencing, and reduced IQ. Specific cognitive domains most affected were perceptual/non-verbal reasoning, working memory, and attention, with a trend towards executive dysfunction and impaired visuospatial abilities. Verbal paired associate memory

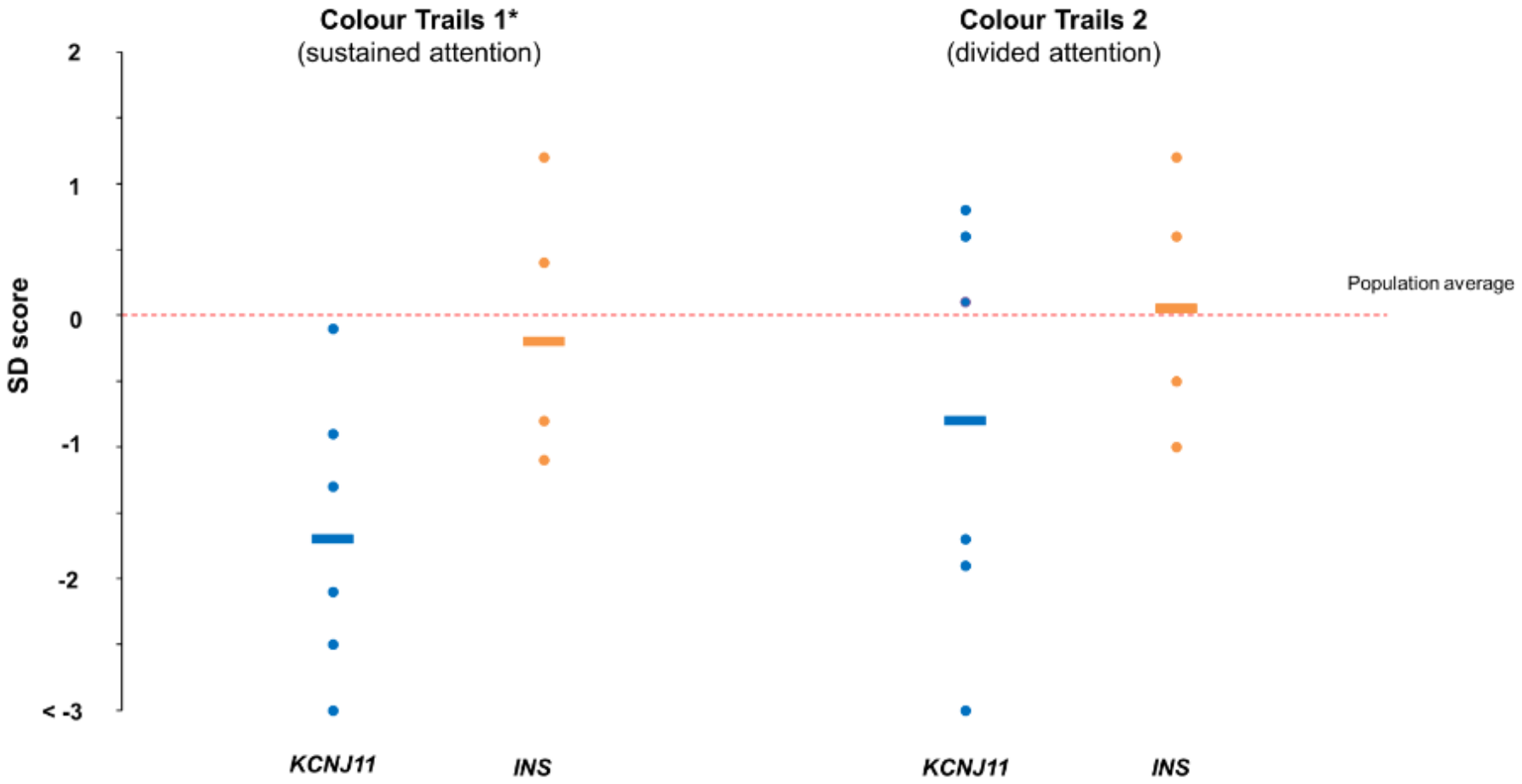

Figure 2: Colour Trails Test 1&2 scores represented as Z-scores in the *KCNJ11* group (blue, n=6) and *INS* group (orange, n=4). Lines show group medians for each subtest; dots represent individuals. *denotes p<0.05 for difference between *KCNJ11* and *INS* groups.

was relatively preserved. The impact on everyday functioning was significant; 2 participants were severely impaired, requiring support with activities of daily living. A comparison group of patients with neonatal diabetes of similar duration due to *INS* mutations did not show any of these specific features indicating that they are unlikely to be a non-specific effect of metabolic disturbance from birth. Furthermore, as both groups were insulin treated from diagnosis as infants, the differences observed are unlikely to have been influenced by variation in the timing or duration of action of insulin on insulin and IGF receptors in the brain.

Our findings are consistent with studies in paediatric cohorts with *KCNJ11* neonatal diabetes. Specifically, the motor features noted on neurological examination, together with impairments of attention, working memory, visuospatial ability and executive function are consistent with the previously reported high prevalence of developmental coordination disorder, inattention, executive dysfunction and poor visuomotor performance in children with *KCNJ11* mutations (12-14; 17; 18; 23; 40). ASD features in 4 individuals with *KCNJ11* mutations is consistent with previous research reporting high rates of neurodevelopmental disorders in affected children (16; 18; 41). Dyspraxia, visuomotor impairment, autism and impaired executive function may be related to the high levels of expression of dysfunctional K_{ATP} channels in the cerebellum in *KCNJ11* PNDM (7; 10).

Importantly, the abnormal findings we report in the *KCNJ11* group were mutation-specific; the 2 individuals with the R201H mutation had no overt features and only some subtle abnormalities on neuropsychological testing (Table S2). As a result,

both were able to live independently and support themselves financially. Those with more severe features required high levels of support from family members and professionals in healthcare, education and social care. This is consistent with previous studies showing the severity of the CNS phenotype is related to the specific mutation; for example, the V59M mutation results in more severe features, greater impairment in daily living skills (13) and greater impact on families (16). Interestingly, however, there was a relatively good level of social integration from all patients with *KCNJ11* mutations even when the neurobehavioral features were severe.

Some of our findings contrast with previous research. To our knowledge choreiform movements have not been previously associated with *KCNJ11* PNDM but were observed in one individual with the V59M mutation in our study. We did not identify abnormal tone in our cohort, which contrasts with the hypotonia previously reported, particularly in the context of DEND/iDEND syndrome (42). This may be explained by 7/8 individuals in our study being sulfonylurea-treated; improvement in tone to near-normal following transfer from insulin to sulfonylureas has been observed in a recent study of children with *KCNJ11* PNDM (23). Similarly, improvement of visuospatial abilities and attention following transfer to sulfonylureas was noted in the paediatric study (23), which could account for the attention deficits and visuospatial impairment being less marked in our cohort of sulfonylurea-treated adults than might have been expected given previous descriptions (17; 23). Our neuroimaging findings contrast with this paediatric study in which there were non-specific findings in 12/17 who underwent brain MRI, largely comprising white matter abnormalities (23). However, these scans were performed at baseline prior to transfer to

sulfonylureas (23). It is not known whether the abnormalities would have improved after a period of sulfonylurea treatment, as has been shown in SPECT studies (24; 27).

Sulfonylurea treatment may influence the CNS phenotype in *KCNJ11* PNDM. Two studies have suggested that an earlier age of initiation of sulfonylureas can lead to better CNS outcomes (17; 23). We were unable to assess this in our study because median age at transfer to sulfonylureas was 18 years (range 11-34). However, the persistence of CNS features in some patients even after early initiation of treatment (16; 23) suggests other factors are involved. Specifically, active transport of glyburide out of the brain across the blood-brain barrier, as has been demonstrated in a rodent model (28), may result in suboptimal concentrations in the CSF, thereby limiting therapeutic efficacy in the human CNS. Anecdotal clinical experience suggests that this can be partially addressed by increasing the dose of glyburide to ~1mg/kg/day; however there have been no cases of complete resolution of CNS features in a patient with iDEND. Another possible reason for the partial response is that pathways that can fully restore K_{ATP} channel function in other tissues are not available in the CNS to interact with brain K_{ATP} channels. For example, restoration of pancreatic K_{ATP} channel function resulting in excellent glycaemic control with sulfonylurea treatment is dependent on the activity of incretin hormones (3). Furthermore, there is a theoretical impact of insulin deficiency in utero and / or C-peptide deficiency prior to sulfonylurea transfer on the brain as an indirect consequence of *KCNJ11* mutations, but more studies are needed to explore this in humans. Indeed, given the complexities of human neurodevelopmental processes, it is likely that several

factors contribute in some way to the response of CNS features to sulfonylureas in *KCNJ11* PNDM.

Strengths, Limitations and future work

This study has important strengths. It is the first to assess in detail the CNS manifestations of *KCNJ11* mutations in adults and to control for the non-specific effects of PNDM by comparing the features in individuals with *INS* mutations. Limitations of the study, which relate to the rarity of the disease, are the small number of individuals in each group, the broad range of mutations studied, and the variable timing of initiation and duration of treatment with sulfonylureas in the *KCNJ11* group. Studies in larger cohorts with single specific mutations would be valuable. Furthermore, exploration of the impact of treatment-specific factors, such as age of initiation, dose and CNS handling of sulfonylureas in humans, on CNS features in *KCNJ11* PNDM is warranted.

Conclusion

The CNS phenotype in adults with *KCNJ11* mutations comprises learning difficulty, autistic features, subtle motor dysfunction, moderately reduced IQ, and impaired attention, perceptual reasoning and working memory. The severity of these features varies with the causative mutation. They persist despite long term sulfonylurea therapy, at least when this is started after the first decade of life, and represent the major burden from *KCNJ11* mutations once glycemia is well controlled on sulfonylureas. These CNS features are not present in individuals with *INS* mutations, which indicates that they do not occur as a result of the lifelong metabolic disturbance imposed by PNDM, but as a consequence of

impaired K_{ATP} channel function in the brain. Clinicians in adult and paediatric medicine should be aware of the potential impact of CNS features in patients with *KCNJ11* mutations and should consider multidisciplinary management to ensure appropriate support is provided.

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Conflicts of interest disclosures

None to declare.

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Supplementary material

Diabetes Care 2019 42(2):215-224

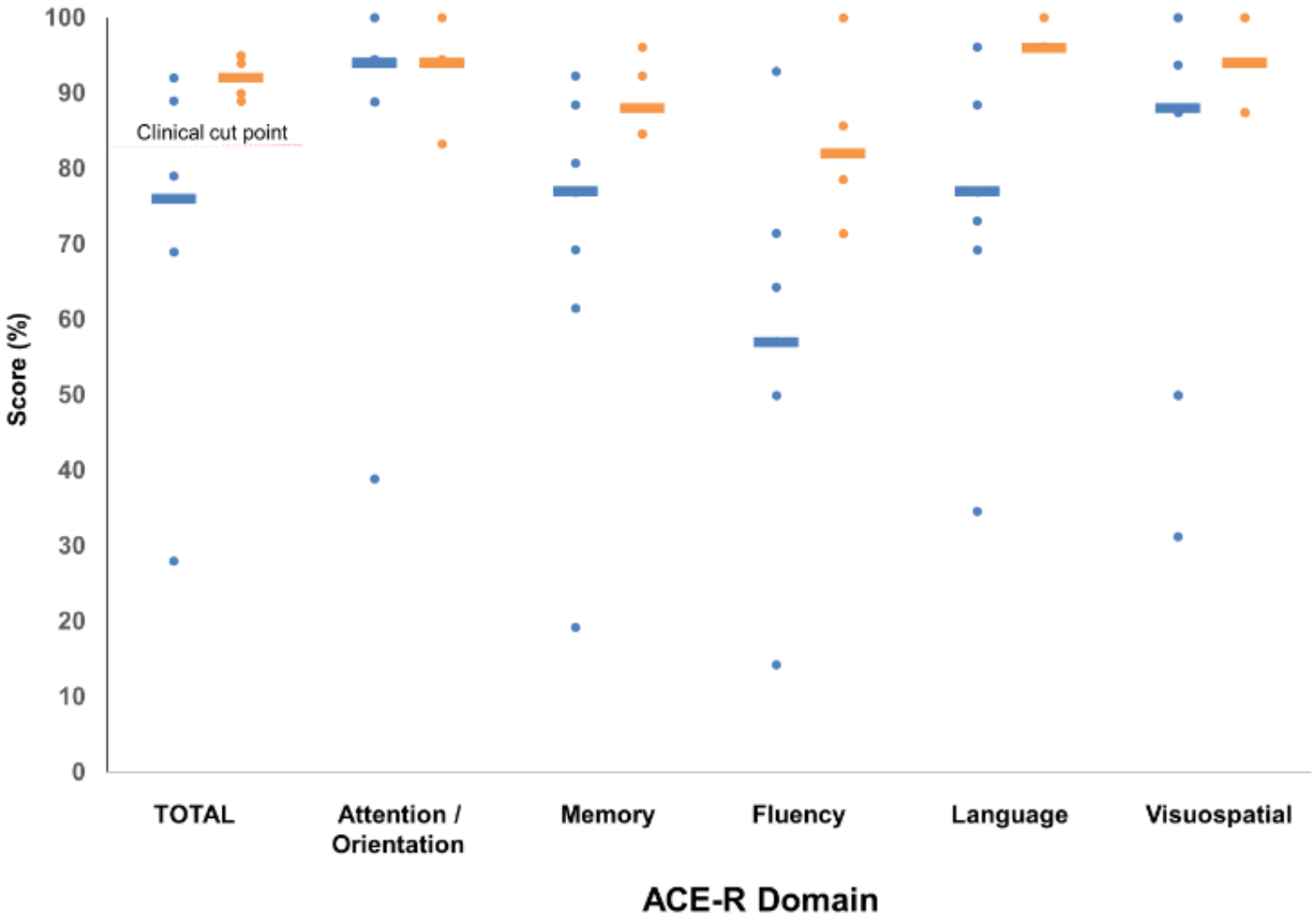

Figure S1; ACE-R in KCNJ11 individuals (blue), n=7 and INS individuals (orange), n=4. Scores are presented as percentages of maximum possible score for each subsection. A total score of ≤82 is suggests clinically relevant cognitive impairment.

Table S1 – Clinical characteristics of participants with *KCNJ11* mutations excluded from analysis

Case ID (mutation)	Sex	Age (years)	Age at diabetes diagnosis (weeks)	Age at genetic diagnosis (years)	Age at SU transfer (years)	Treatment (total daily dose)	Development (type of delay)	Seizures (cause, if known)	Learning difficulties / support / education	Employment status (job)	Psychiatric history	Neurological features
KCNJ11 9 (V59M)	F	44	8	35	36	Glyburide (60mg)	D (global)	Yes – hypoglycaemia on insulin	Unable to speak / read / write, SS	UE	Nil	Spastic tetraparesis, able to follow 1-step commands only, unable to speak but able to vocalise noises.
KCNJ11 10 (L164P)	M	44	16	39	40	Glyburide (dose NK)	D (motor)	No	LS (through school), MS	MR (previously E - Post Office)	Bipolar disorder – two hospital admissions. On ziprasidone, fluoxetine, metoclopramide.	Cogging pursuit eye movement, hypomimia, reduced arm swing, cogwheel rigidity of arms, mild rigidity of legs. Difficulty with motor sequences.

NK = not known, D=delayed, MS = Mainstream school, SS = Special school, LS = learning support, E = employed, UE = unemployed, MR = medically retired

Table S2 - Individual scores on subtests of the Wechsler Memory Scale 4th edition (WMS-IV), Wechsler Abbreviated Scale of Intelligence (WASI), Wechsler Adult Intelligence Scale 4th edition (WAIS-IV), and Colour Trails Test (CTT) I and II. ND = Not done.

Patient	Mutation	WMS-IV (Z-score)				WASI (Z-score)				WAIS-IV (Z-score)		Colour Trails Test	
		Verbal paired associates (immediate)	Verbal paired associates (delayed)	Visual reproduction (immediate)	Visual reproduction (delayed)	Vocabulary	Matrix reasoning	IQ (raw score)	IQ (Z-score)	Digit span	Cancellation	CTT 1	CTT 2
KCNJ11 1	G53S	-1.67	-2	-3	-1.67	-2.3	-1.8	65	-2.33	-2.33	-1	-2.5	-1.7
KCNJ11 2	R201H	0	0.33	0	0.33	0.5	-2.3	101	0.067	0.33	0	-0.9	0.8
KCNJ11 3	R201H	0.33	1	0.67	1.33	1.6	-3.2	99	-0.067	-1.33	0	-0.1	0.6
KCNJ11 4	R201C	0	0.33	-1.33	-1.33	-0.9	-0.9	86	-0.933	-2	-1	-1.3	0.1
KCNJ11 5	R201C	-1.67	-1.67	-1.33	-0.33	0.1	-4	76	-1.6	-1.67	-1	-2.1	-3
KCNJ11 6	V252G	-2	-2.67	-3	-3	-3.2	-4.8	55	-3	-3	-3	ND	ND
KCNJ11 7	V59M	0	0.66	-3	-2.33	-1.1	-4.4	65	-2.33	-3	-2	-3	-1.9
KCNJ11 8	V59M	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
INS 1	C43F	0.67	1.33	0.33	1	2.1	0.6	124	1.6	0.33	2.67	0.13	0.6
INS 2	F48C	1.67	1.33	0.67	0	0.9	0.6	113	0.867	0	3	0.6	0.8
INS 3	G75C	-1	-0.67	1	0	0.2	0.8	109	0.6	-1	3	1.2	1.2
INS 4	H29D	0	0	-1.33	-0.67	-0.4	-0.7	90	-0.67	0	0.67	-1.1	-1

Table S3 - Individual scores on Autism Spectrum Quotient (AQ), Hospital Anxiety and Depression Scale (HADS), Controlled Oral Word Association Test (COWAT), and subtests of the Visual Object and Space Perception battery (VOSP) and Addenbrooke's Cognitive Examination-Revised (ACE-R). ND = Not done.

Patient	Mutation	AQ raw score	HADS raw score		COWAT Z-score	VOSP Score (Pass/Fail)				ACE-R raw score					
			HADS A	HADS D		Incomplete Letters	Object Decision	Dot Counting	Cube Analysis	Total (/100)	Attention / orientation (/18)	Memory (/26)	Fluency (/14)	Language (/26)	Visuospatial (/16)
KCNJ11 1	G53S	33	9	1	-2.21	F	P	P	F	69	16	20	7	18	8
KCNJ11 2	R201H	12	6	6	-1.31	P	P	P	P	89	17	23	10	23	16
KCNJ11 3	R201H	17	9	2	0.79	P	P	P	P	92	18	21	13	25	15
KCNJ11 4	R201C	32	9	7	-1.91	P	F	P	P	76	18	16	8	20	14
KCNJ11 5	R201C	24	10	5	-1.72	P	F	P	F	79	16	18	9	20	16
KCNJ11 6	V252G	ND	14	8	ND	P	F	F	F	28	7	5	2	9	5
KCNJ11 7	V59M	25	9	7	-1.92	P	P	F	F	76	17	24	8	19	8
KCNJ11 8	V59M	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
INS 1	C43F	5	1	0	-0.77	P	P	P	P	95	18	24	11	26	16
INS 2	F48C	15	12	4	-0.86	P	P	P	P	89	15	25	10	25	14
INS 3	G75C	26	6	1	-0.42	P	P	P	P	94	17	22	14	25	16
INS 4	H29D	30	17	7	-0.79	P	F	P	P	90	17	22	12	25	14

CONCLUSIONS

CHAPTER 1

Summary

Chapter 1 is an international cohort study that followed 81 patients with *KCNJ11* PNDM over a median of 10.2 years and assessed the efficacy and safety of sulphonylurea treatment, as well as the impact on neurological features.

Conclusions

The study showed that sulphonylureas are highly effective, with 93% patients remaining independent of insulin at most recent follow-up and maintaining excellent glycaemic control (median HbA1c 6.4% (46.4mmol/mol) at 10 years). Importantly, sulphonylureas are safe and do not result in severe hypoglycaemia or significant side-effects, even at doses 2-10 times greater than those used in Type 2 Diabetes. If present, neurological features persist in sulphonylurea-treated *KCNJ11* PNDM long-term, although there is partial improvement in these features in ~half of affected patients on transfer from insulin to sulphonylurea therapy. This research confirms that sulphonylureas are the treatment of choice in *KCNJ11* PNDM and emphasises the importance of prompt genetic testing of all individuals who develop diabetes in the first 6 months of life, to facilitate rapid transfer from insulin to sulphonylureas in those with *KCNJ11* mutations.

Impact

This research has significant implications for individuals with *KCNJ11* PNDM and their clinicians, as it is the only study to address the question of long-term outcomes of sulphonylurea treatment. The study has been acknowledged in a recent review article as being of major importance in the field (1). It provides reassurance that sulphonylureas continue to work extremely well in achieving

outstanding metabolic control for at least 10 years in *KCNJ11* PNDM with no safety concerns. Clinicians can therefore be confident in prescribing high dose sulphonylureas for patients with *KCNJ11* PNDM and can provide accurate counselling in relation to the predicted long-term outcomes of this treatment. For affected individuals and the parents of children with *KCNJ11* PNDM, the knowledge that sulphonylureas are the optimum therapy for the condition is likely to provide peace of mind and potentially improved quality of life. This is illustrated by responses from affected individuals and families when they were told the outcomes of the study:

“Amazing to think that 10 years ago I was asked to do test for which I was told would change my diabetes care. In which changed my course of treatments from insulin to sulphonylureas which in turn amazingly changed my life from day to day worries of blood glucose and insulin injection time to never worrying about have hypoglycaemia attack . And that the length time I've been on sulphonylureas gives hope to all those young and old that the team at Exeter are able to treat like me.” (Patient GR)

"When S first transitioned from insulin injections over ten years ago we were amazed by the improvements in blood sugar control and the greater independence this brought. Life became more flexible, less of a worry and no longer a juggling act between measuring food intake against judging the insulin required. Knowing sulphonylureas is a 'fix for life', has had huge positive impact on the whole family." (parents of patient ST)

The study also has wider implications. Whilst many examples of precision medicine result in good short-term outcomes, treatment failure is common in the medium-long term particularly in fields such as oncology where, over time, cancers are able to acquire new mutations that confer resistance to

pharmacological therapies (2). In the case of *KCNJ11* PNDM, there is a fixed genetic defect that does not change over time, and therapy targeted to this specific defect results in excellent clinical outcomes for at least 10 years. Sulphonylurea-treated *KCNJ11* PNDM therefore represents an excellent example of precision medicine and demonstrates how effective specific targeted treatments can be in monogenic disease.

Future Research

It will be crucial to continue to follow the progress of large cohorts of patients with sulphonylurea-treated *KCNJ11* neonatal diabetes beyond 10 years, with longitudinal assessments of glycaemic and neurological outcomes, to investigate longer-term durability of sulphonylurea therapy. More in-depth neuropsychological, psychiatric and behavioural assessments undertaken at multiple time points throughout life will be important to map the neurodevelopmental trajectories of affected individuals, and to provide more information on the impact of specific genetic variants as well as sulphonylurea treatment.

In addition, the effects of specific physiological states e.g. puberty, pregnancy, on metabolic control and sulphonylurea efficacy will require investigation, particularly as clinical experience suggests that dose requirements increase during puberty. The use of cell-free fetal DNA (cffDNA) for in utero diagnosis of monogenic diabetes (3) will be extremely useful in identifying cases for prospective follow-up at an early stage of development, in those families with a history of neonatal diabetes. Furthermore, sulphonylurea therapy is recommended in mothers with K_{ATP} channel mutations when the variant has been inherited by the fetus (4), and it will be important to assess the impact of *in*

utero sulphonylurea treatment on both insulin-mediated growth and neurodevelopment.

The influence of genetic factors in relation to research into sulphonylurea handling and bioavailability should also be considered. For example, there is evidence from the T2D literature that genetic variants in cytochrome P450, family 2, subfamily C, polypeptide 9 (*CYP2C9*) can influence the rate of metabolism of sulphonylureas, with specific alleles resulting in reduced clearance, longer plasma half-life and higher AUC (5). An impact of *CYP2C9* on drug response has also been reported. Specifically, carriers of the *CYP2C9*2* or *CYP2C9*3* variants, which have a loss-of-function effect, had a 0.5% larger reduction in HbA1c than wild-type carriers in a retrospective study of over 1000 individuals with T2D resulting in greater likelihood of achieving therapeutic targets for glycaemic response (6). Furthermore, interactions between these variants and *POR*28*, a variant in the P450 oxidoreductase gene, have been shown to result in improved response to sulphonylureas but with greater odds of hypoglycaemia in T2D (7). Such studies suggest that genetic factors other than the primary mutation in *KCNJ11* might have the potential to affect sulphonylurea treatment response in people with neonatal diabetes, but small cohort sizes have limited the ability to formally test this. As research cohorts increase in size in the future, there may be sufficient power to conduct pharmacogenetics studies specific to *KCNJ11* neonatal diabetes.

Qualitative research with patients with *KCNJ11* mutations represents another key future direction. A hugely positive impact on quality of life was reported by affected patients and families in the short-term following transfer from insulin to sulphonylureas (8). Initial responses from patients and families suggest that this is also the case long-term (see quotes above), but it will be important to

establish this formally, by undertaking interviews and focus groups exploring the impact of sulphonylurea treatment. Also of interest will be qualitative work with those few individuals with *KCNJ11* PNDM who do require reintroduction of insulin, to explore the biopsychosocial factors that may contribute to treatment failure.

Further research in related genes and phenotypes and comparison of these with *KCNJ11* PNDM will also be important to inform future clinical guidelines relating to K_{ATP} channel neonatal diabetes. The *ABCC8* gene encodes the regulatory subunits of the K_{ATP} channel and contains the binding site for sulphonylureas (9). *ABCC8* mutations are the second commonest cause of permanent neonatal diabetes (10) and 85% of patients can be treated with sulphonylureas in the short-term (11). Clinical experience and preliminary research evidence (Bowman, unpublished) suggests that the long-term response to sulphonylureas is also excellent in this group of patients. However, detailed analysis of long-term outcomes in sulphonylurea-treated *ABCC8* PNDM and comparison with those reported in *KCNJ11* PNDM is crucial to expand the evidence base and inform clinical management.

Similarly, patients with transient neonatal diabetes (TNDM) and adult-onset diabetes due to *KCNJ11* or *ABCC8* mutations, represent an understudied yet fascinating group in which sulphonylurea therapy is effective (11); lower doses are recommended for individuals with TNDM mutations than those required for K_{ATP} channel PNDM (12, 13). Longitudinal research including physiological studies during remission and relapse are needed to address key clinical and mechanistic questions relating to the role of pharmacological, genetic and environmental factors in the pathophysiology and natural history of K_{ATP} channel TNDM.

CHAPTER 2

Summary

Chapter 2 is a physiological study that assesses the insulin, glucose and glucagon response to carbohydrate and protein/fat in patients with sulphonylurea-treated *KCNJ11* PNDM and non-diabetic controls. The study also assesses the response in the absence of food (with sulphonylurea only) in those with *KCNJ11* PNDM.

Conclusions

People with sulphonylurea-treated *KCNJ11* PNDM have similar insulin secretion after a protein/fat and carbohydrate meal, despite lower glucose values after a protein/fat meal and higher glucose values after a carbohydrate meal. This is in contrast to non-diabetic controls in whom insulin secretion is greater after a carbohydrate meal than after a protein/fat meal, and in whom glucose remains tightly controlled after both meals. The relatively low glucose values after protein/fat in individuals with *KCNJ11* PNDM may put them at risk of post-prandial hypoglycaemia following meals lacking carbohydrate.

The results emphasise the lack of response to glucose and predominance of non-K_{ATP}-channel pathways of insulin secretion (e.g. incretins, direct effect of amino acids and fatty acids) in the context of sulphonylurea-treated *KCNJ11* PNDM, whilst non-diabetic individuals remain glucose-responsive with insulin secretion driven primarily through K_{ATP}-channel pathways. Consistent with a dependence on food for insulin secretion, insulin levels in the absence of a meal are lower in individuals with sulphonylurea-treated *KCNJ11* PNDM. However, glucose levels in this situation are also relatively low, suggesting there is some direct effect of the sulphonylurea drug in the absence of food. In both cases

and controls, glucagon secretion was higher after a protein/fat meal than after a carbohydrate meal, supporting the direct stimulation of alpha cells by amino and / or fatty acids.

Impact

This research is impactful for clinicians and patients with sulphonylurea-treated *KCNJ11* PNDM as it can be used to inform the clinical care of affected individuals. We observed a fall in glucose after a protein/fat meal and with no meal at all (sulphonylurea only). Clinical recommendations therefore include ensuring all meals contain some carbohydrate, and not missing meals whilst taking sulphonylureas.

The study also has impact in terms of providing a mechanistic explanation as to why people with sulphonylurea-treated *KCNJ11* PNDM can experience mild-moderate hypoglycaemia after meals lacking carbohydrate. In the presence of a mutation, which confers insensitivity to rising or falling ATP, there is a relative inability to 'switch off' insulin secretion in response to falling glucose after protein/fat meals. Understanding mechanism is helpful for clinicians and patients but also for the scientific community studying this and related fields.

More broadly, this research demonstrates how individuals with *KCNJ11* mutations can be used as a human model to study non-K_{ATP}-mediated pathways of insulin secretion. This may have relevance for other forms of diabetes where such pathways represent known and potential drug targets.

Future Research

Future directions of study will include replication of this research in paediatric cohorts, in whom there will be a shorter duration of diabetes and an earlier age of sulphonylurea initiation than in adults; it is not known if this affects beta cell

function in the context of different food stimuli. It will be also be important to conduct further research into hypoglycaemia and counter-regulatory responses in individuals with sulphonylurea-treated *KCNJ11* PNDM through further physiological studies as well as pragmatic clinical studies.

The lack of severe hypoglycemia despite very high doses of sulphonylurea is not yet fully understood. K_{ATP} channels are present on glucagon-secreting alpha cells (14) and in the brain (15). It is thought that both have roles to play in counter-regulation, although the exact function of K_{ATP} channels in this context remains uncertain (16, 17). Future research will investigate these counter-regulatory responses in detail via hypoglycaemic clamp studies.

Furthermore, the effects of sulphonylurea dose, timing of treatment, and exercise on the risk of mild-moderate hypoglycaemia is not known. These are key clinical questions as they have implications for the treatment and lifestyle advice offered to patients with this condition. Importantly, such issues have been specifically raised by the patients themselves during their interactions with clinicians and researchers. Future studies will address these questions in both controlled laboratory conditions and in the 'real-life' context e.g. via the use of relatively non-invasive methods such as flash glucose monitoring (FGM) (18).

Another important line of research for the future will be further assessment of the role of incretin hormones in the context of sulphonylurea-treated *KCNJ11* PNDM. The evidence to date suggests that this pathway remains intact and indeed predominates in affected individuals. The only study to measure the glucagon-like peptide-1 (GLP-1) response to oral glucose in 4 patients with *KCNJ11* PNDM showed similar levels of the hormone before and after sulphonylurea transfer (19). However, no studies have measured gastric inhibitory polypeptide (GIP) or assessed the impact of different types of food on

incretin hormone secretion in these patients. In addition, it would be of interest to investigate the impact of pharmacological augmentation of the incretin pathway through the administration of GLP-1 receptor agonists, GLP-1 analogues or DPP-4 inhibitors. In the few individuals with *KCNJ11* PNDM whose glycaemic control on sulphonylureas in suboptimal, this may represent a logical next step in treatment, although it would also be important to establish whether the risk of hypoglycaemia would be increased by enhancing stimulation of the incretin pathway in the absence of moderation by ATP.

CHAPTER 3

Summary

The chapter comprises 2 parts which describe the psychiatric and neuropsychological manifestations of *KCNJ11* mutations in children with sulphonylurea-treated *KCNJ11* neonatal diabetes (9 PNDM and 1 TNDM). In part A the psychiatric morbidity is explored using validated questionnaires and compared with UK school-age population norms. In part B a battery of specific standardised neuropsychological tests is used to assess cognitive performance across several key domains and outcomes in affected individuals are compared with their unaffected siblings.

Conclusions

In part A, we showed that there is significant psychiatric morbidity in children with sulphonylurea-treated *KCNJ11* neonatal diabetes, particularly in association with specific mutations e.g. V59M, R201C. Our data support at

least one psychiatric diagnosis in 6/10 children tested, which is significantly higher than that seen in school-age children in the general population (10% using the same standardised assessments). The types of disorders observed in children with *KCNJ11* mutations are predominantly neurodevelopmental e.g. autism, ADHD, but anxiety disorders are also frequently seen. Despite SDQ scores indicating high impact of these disorders at home and at school, many are unrecognised clinically, with only 2/14 diagnoses having been made in clinical practice.

In part B, we showed that in children with *KCNJ11* neonatal diabetes who do not have mutations known to cause DEND or iDEND, there are cognitive impairments in comparison to non-diabetic siblings that can be identified using standardised neuropsychological tests. These affect a range of domains but particularly executive function, verbal comprehension and visuomotor performance. This is consistent with what has been reported elsewhere in the literature (20-22) and supports early and comprehensive neuropsychological testing in individuals with *KCNJ11* neonatal diabetes even in the absence of overt severe neurological features.

Impact

Both studies in this chapter have scientific and clinical impact through expanding our knowledge of the CNS manifestations of *KCNJ11* mutations. Part A is the first study to formally assess and describe the psychiatric morbidity in children with sulphonylurea-treated *KCNJ11* neonatal diabetes, and the high impact this has on patients' lives, particularly in association with specific genotypes e.g. V59M. The findings have recently been supported in an

observational study that used the SDQ to assess 8 children with *KCNJ11* neonatal diabetes (23).

Part B provides additional evidence for the presence of specific neuropsychological impairments in children with *KCNJ11* mutations who do not have the severe DEND / iDEND phenotype.

This research is of importance for the clinical care of affected individuals and their families. Specifically, healthcare professionals are now better placed to counsel families regarding the neurological aspects of this condition, which frequently represent the greatest challenge given the excellent glycaemic control obtained on sulphonylurea therapy. Furthermore, clinicians can refer affected patients to the appropriate specialists for neuropsychological testing, clinical diagnostic assessments and ongoing support. Ultimately this will result in joined up multi-professional care for children with *KCNJ11* PNDM so that the metabolic and neurological aspects of their condition can be treated, and appropriate educational interventions provided as early as possible. In-keeping with this, some individuals have sought diagnostic clinical assessments following participation in this research or been able to use the results of the research to obtain improved educational support.

Future Research

The studies in this chapter provide a crucial foundation for future research both clinically, in terms of further exploration of genotype-phenotype relationships and development of improved treatments for the neurological sequelae, and mechanistically, in terms of investigating the role of the K_{ATP} channel in the brain.

Most of the children assessed in these studies had one of the commoner mutations seen in *KCNJ11* neonatal diabetes e.g. V59M, R201H, R201C. However, some had rarer mutations which make the interpretation and generalisability of the findings relating to CNS features more challenging. Future studies in larger cohorts containing more patients with each genetic variant will be required to further develop our understanding of how specific genotypes influence the neurological phenotype and the possible reasons for this. In addition, the impact of TNDM mutations on the CNS can be explored. Only one patient assessed in our cohort had TNDM and this individual had mild speech delay and required learning support. Other studies have also suggested the presence of neuropsychological impairments in association with TNDM mutations (21), but this has not been assessed in detail and will be an important future direction for research. Similarly, the impact of *ABCC8* mutations causing either PNDM or TNDM on CNS functioning requires investigation in larger cohorts of affected patients. An interesting research question is whether there are any specific differences in the neurological phenotype and treatment response between patients with neonatal diabetes due to *KCNJ11* vs *ABCC8* mutations.

Although glibenclamide treatment can result in partial improvement of the neurobehavioural features in *KCNJ11* neonatal diabetes (24), our studies and others show that such features persist in children on sulphonylurea treatment and indeed represent a significant disease burden. Many research questions about the impact of sulphonylurea treatment on the CNS remain unanswered. Firstly, the penetration of sulphonylureas across the blood-brain barrier (BBB) has not been studied in humans. In rats, glibenclamide crosses into the brain and is rapidly pumped back out across the BBB such that therapeutic

concentrations of the drug are not achieved in the cerebrospinal fluid (CSF) (25). Currently clinicians are advised to prescribe larger doses (~1mg/kg/day glibenclamide) in the presence of severe neurological features, but this is based on research in rodents and anecdotal clinical evidence of improvements in humans on such high doses. Future research will focus on measurement of sulphonylurea levels in human CSF and how these relate to blood levels, for glibenclamide and other sulphonylureas. It will be important to also consider the effects of pharmacogenetics in relation to this, as described for study 1. Such research will provide an evidence base to help inform prescribing in clinical practice in individuals with *KCNJ11* neonatal diabetes, particularly if there is a significant neurological component to the presentation.

Another factor that may play an important role in the neurological response in *KCNJ11* PNDM is the age of initiation of sulphonylurea treatment. Some preliminary research evidence in small cohorts of patients with *KCNJ11* mutations (one cross sectional and one prospective study) has suggested that earlier treatment results in better CNS outcomes (24, 26). This may relate to increased neuroplasticity in the younger brain and therefore an ability to recover greater function if K_{ATP} channels are targeted at a very early stage of development e.g. within the first 6 months of life, a so-called 'sensitive period' (27). Such an hypothesis is supported by neurodevelopmental studies in Romanian adoptees; long-term follow-up showed that those children who experienced severe deprivation due to institutionalisation for greater than 6 months had high rates of autism, inattention, overactivity and disinhibited social engagement which persisted through childhood into early adulthood. In contrast, children adopted under 6 months of age had lower rates of all disorders at all ages and these were comparable to rates observed in a UK

adoptee control group (28, 29). Although one recent cross-sectional study did not find a significant correlation between the age of initiation of sulphonylureas and neuropsychological outcomes in patients with *KCNJ11* neonatal diabetes, this was based on an analysis of only 5 patients and a 6 month cut-off for treatment initiation was not applied (23). Future studies will focus on the neurodevelopmental outcomes in larger cohorts of patients treated with sulphonylureas in the first 6 months of life and will compare these with the outcomes in those who transferred later.

Furthermore, recent advances in laboratory techniques have resulted in the use of cell free fetal DNA (cffDNA) to detect neonatal diabetes-causing mutations in utero in those fetuses with an affected parent (3). Glibenclamide crosses the placenta and is detectable in umbilical venous blood after delivery (30), although thus far studies have been done in women with gestational diabetes (GDM) who would be on lower doses of glibenclamide than those with *KCNJ11* PNDM. Using cffDNA, there will be opportunities in the future to identify affected fetuses early and assess the impact of glibenclamide treatment during pregnancy if they have inherited a *KCNJ11* mutation from their mother.

Finally, there have been no clinical trials assessing the efficacy of other drugs targeted to brain K_{ATP} channels. Carbamazepine inhibits K_{ATP} channels *in vitro* via a similar mechanism to sulphonylureas (31) and is already used as an anti-epileptic drug in clinical practice. Memantine inhibits hippocampal K_{ATP} channels expressing Kir6.2 and this is thought to be a major mechanism by which the drug has therapeutic efficacy in Alzheimer's disease (32). It will be important to explore in future research studies whether repurposing of these drugs can provide benefits for individuals with *KCNJ11* mutations in addition to those observed with sulphonylurea treatment.

CHAPTER 4

Summary

This chapter is the first study to assess in detail the neurological, neuropsychological and behavioural profile of adults with *KCNJ11* PNDM (n=8), and to compare this with adults with diabetes from birth due to *INS* mutations (n=4), thereby controlling for metabolic disturbance from an early age.

Conclusions

Adults with *KCNJ11* mutations have a range of neurobehavioural and neuropsychological features that persist with sulphonylurea treatment and have a significant functional impact on activities of daily living. Specific features identified in this study were similar to those observed in children with *KCNJ11* neonatal diabetes and include autistic traits, reduced IQ, and impaired attention, reasoning and working memory. There was also impaired motor sequencing and coordination, and a trend towards impaired visuospatial and executive functions. The severity of these features related to the genotype, with most difficulties associated with the V59M and R201C mutations. The impairments observed in the *KCNJ11* group were not present in individuals with *INS* mutations, suggesting they are directly related to the genetic abnormality as opposed to a consequence of the early and ongoing metabolic disturbance(s) associated with diabetes.

Impact

This research is impactful in that it enhances our understanding of the nature and aetiology of CNS features in *KCNJ11* neonatal diabetes. The presence of CNS features in the individuals with *KCNJ11* mutations but not *INS* mutations supports a direct role of dysfunctional K_{ATP} channels in the brain as opposed to

a non-specific effect of hyperglycaemia from birth. The specific impairments observed are different to those seen in patients with K_{ATP} channel neonatal diabetes who suffered cerebral oedema secondary to diabetic ketoacidosis at initial presentation (33). Furthermore, both the *KCNJ11* and *INS* groups were treated with insulin from diagnosis as infants (until successful transfer to sulphonylureas in 7/8 *KCNJ11* patients at a median age of 21 years); both genetic subtypes are insulin deficient and require replacement doses. Therefore, variation in the action of insulin on brain insulin and insulin-like growth factor (IGF) receptors would not explain the presence of specific defects in the *KCNJ11* group and not in the *INS* group.

Future Research

Future studies in this area will assess the longitudinal progression of the CNS features in adults with *KCNJ11* mutations and the impact of these mutations in older adults. In people with T1D neuropsychological impairments show progression over time (34). In those with T2D, cognitive deficits are more pronounced in individuals aged >60-65 years (35) and there is an increased risk of dementia with poor metabolic control accelerating the rate of cognitive decline; specific domains affected include executive function, learning and memory, psychomotor speed and attention (36). This has a significant impact on daily life including the ability to adhere to medication regimes and function independently (37). Although the pathophysiology and treatment response in K_{ATP} channel neonatal diabetes is clearly different from T1D and T2D, the extent to which the CNS features in people with *KCNJ11* mutations progress throughout adult life is not known. Longitudinal follow-up studies with repeated testing are needed to assess this. In addition, it would be valuable to perform health economic analyses as part of future research. Current estimates of the

cost saving made per individual on switching treatment from insulin to sulphonylureas in *KCNJ11* neonatal diabetes are in the region of $30,000 over 30 years (38). However, this model is based on the American healthcare system and additional studies are needed to accurately identify the cost effectiveness of genetic diagnosis and treatment change within the UK National Health Service (NHS).

Further future directions for research will include the areas outlined above for chapter 3: the impact of sulphonylurea-related factors (age of initiation, dose, type, BBB permeability) and the potential repurposing of other drugs that act on CNS K_{ATP} channels are all relevant for adults with *KCNJ11* mutations as well as children. Furthermore, pancreatic physiology in *KCNJ11* neonatal diabetes relies predominantly on non-K_{ATP} channel amplifying pathways such as incretin hormones; to what extent such pathways are used in the brain is not known. GLP-1 receptors are present in the brain and have been associated with neuroprotection and improved learning and memory in rats (39). Interestingly, the neuroprotective role of GLP-1 has also been observed in humans with neurodegenerative disorders but no diabetes treated with GLP-1 agonists suggesting a glycaemia-independent effect (40). It will be important to consider the potential for drugs targeted at GLP-1 pathways in the brain to be trialled in people with *KCNJ11* mutations.

FINAL REMARKS

Summary and dissemination of research findings

The research described within this thesis has advanced knowledge relating to both the glycaemic and CNS response to sulphonylureas in people with *KCNJ11* mutations. We have shown that sulphonylureas are a very effective and safe long-term treatment in individuals with *KCNJ11* permanent neonatal diabetes, who maintain excellent glycaemic control for at least 10 years without severe hypoglycaemia. The key pathways for endogenous insulin secretion in this context are non-K_{ATP}-channel mediated amplifying pathways; this differs from individuals without diabetes in whom the classical ATP pathway predominates. In addition we have shown that a range of neurological, neuropsychological, psychiatric and behavioural features are present in individuals with *KCNJ11* neonatal diabetes. The severity of these features is genotype-specific and normal functioning is not fully recovered with sulphonylurea treatment, despite an initial improvement in some patients. For many families, managing the ongoing CNS abnormalities represent the main challenge since metabolic control is optimal following transfer from insulin to sulphonylurea therapy. The findings from all studies in this thesis have been widely disseminated through peer-reviewed publications, oral presentations at national and international conferences, and education events including the Exeter monogenic diabetes symposium for healthcare professionals, genetic diabetes nurse education days and family education days for patients and families with neonatal diabetes.

Translational impact of the research and future clinical care

The studies in this thesis have had clinical impact for both affected families and their clinicians, by providing a greater evidence base for the clinical management of *KCNJ11* neonatal diabetes. This supports UK NHS strategies that aim to improve healthcare for individuals with rare disease by facilitating earlier diagnosis and intervention, improving care coordination and promoting research (41). Specifically, highlighting the existence and impact of the CNS features associated with *KCNJ11* mutations and raising awareness of these amongst clinicians facilitates earlier recognition and intervention. The aim will be to promote better care coordination amongst healthcare professionals which is frequently poor for families affected by rare disease (42). In-keeping with this, in Exeter we are currently developing a specialist national service for patients with K_{ATP} channel neonatal diabetes which will involve multidisciplinary team assessments and integration of findings into comprehensive individualised care plans that will be fed back to local teams for implementation.

However, the provision of clinical interventions is dependent on patients having a genetic diagnosis of K_{ATP} channel neonatal diabetes in the first place, and the efficacy of such interventions is likely to increase the earlier the genetic diagnosis is made. Clinical referrals for genetic testing for monogenic diabetes have increased over time (10) and therefore it will be important to ensure that ascertainment of existing cases and prompt identification of new cases continue to increase in the future. This would be greatly assisted by the incorporation of glucose into the UK newborn screening programme; encouragingly research has shown that glucose is easily detectable on Guthrie cards and is raised even on day 5 of life in individuals with neonatal diabetes (43). Furthermore, the use of targeted next generation sequencing (tNGS) as an alternative to the

traditional Sanger sequencing approach has revolutionised genetic testing in neonatal diabetes by reducing the time and cost involved (44).

Taking these factors into account, the future vision for people with *KCNJ11* mutations will be early, rapid genetic diagnosis, prompt initiation of sulphonylurea therapy, multidisciplinary assessment and ongoing care involving relevant clinical specialties. Importantly, research and education should be integrated into this approach facilitating a translational model whereby research findings are rapidly incorporated into clinical practice resulting in improved patient care.

Modelling precision medicine: implications for monogenic disease

Sulphonylurea-treated *KCNJ11* neonatal diabetes remains the best example of precision medicine in diabetes. It exemplifies the utility and durability of precision approaches in the context of monogenic disease where the specific genetic defect is amenable to targeted treatment with a given drug. Unlike the situation in oncology, where precision approaches often have some initial success but fail long term, the evidence from the studies in this thesis suggest that this is not the case in *KCNJ11* PNDM. This is most likely due to the fixed genetic change in *KCNJ11* neonatal diabetes and the absence of additional acquired mutations which, in cancers, can confer resistance to targeted pharmacotherapy (2). Implementation of precision medicine for other types of monogenic disease has been successful to an extent. For example, in cystic fibrosis specific therapies can be targeted at the various defects in ATP Binding Cassette (ABC) transporters but the efficacy of such drugs is genotype-specific and will therefore only work for a proportion of affected patients (45). A further caveat is cost; novel drug development not only takes a significant amount of

time but the final product is frequently expensive (45) and for this reason may not be offered to all individuals with a given disease in the context of the resource-limited UK NHS. The efficacy of sulphonylureas in *KCNJ11* neonatal diabetes demonstrates how a relatively cheap and widely used class of drugs can be repurposed for specific conditions if they target the correct pathway(s). Furthermore, although genotype can affect the chances of successful transfer from insulin (46, 47), for the 90% who do transfer the efficacy of sulphonylurea therapy does not appear to be dependent on the specific mutation and its effects on protein function i.e. ATP sensitivity or K_{ATP} channel gating. Given the success of the precision approach to treatment in patients with *KCNJ11* mutations, it will be important to consider drug repurposing as well as novel drug development to improve future clinical care in other forms of neonatal diabetes and monogenic disease more generally.

Scientific impact of the research and broader implications

The research in this thesis and the future studies that build upon it can be useful in a much broader context than *KCNJ11* neonatal diabetes alone. This is because we can use what we learn from studying patients with *KCNJ11* mutations to achieve improved understanding of normal physiological mechanisms and apply findings to other conditions with shared biological pathways, both monogenic and polygenic. Animal models have some utility but their generalisability of to human disease is limited for the reasons outlined in Part 2 of the introduction to the thesis. Patients with *KCNJ11* mutations represent a natural experimental human model of monogenic disease; given the tissue expression of the *KCNJ11* gene this provides a unique opportunity to

study the roles of K_{ATP} channel-mediated pathways relating to both the pancreas and the CNS.

Specifically, future research will involve further investigation of the physiology of insulin, glucagon and incretin hormone secretion in patients with *KCNJ11* neonatal diabetes. Pathways associated with these hormones are targets for current and potential drug therapies and dietary interventions in T2D, but many aspects of human physiology remain poorly understood. For example, scientists have as yet been unable to reach a consensus on the regulation of glucagon secretion from alpha cells and the role(s) of K_{ATP} channels in this process (48). Further physiological studies in individuals with *KCNJ11* mutations may allow a more sophisticated understanding of such mechanisms by highlighting the differences in response when K_{ATP} channel pathways are 'knocked out', as is the case in this unique group of patients. An important aspect of such work will be attempting to understand the relative contribution(s) of brain K_{ATP} channels in glucose sensing and counter-regulatory responses to hypoglycaemia, as well as the role(s) of pancreatic K_{ATP} channels on both alpha and beta cells. Gaining a better mechanistic understanding will not only have direct implications for the care of patients with neonatal diabetes but will be valuable for informing research and possibly clinical care relating to common polygenic subtypes of diabetes.

Similarly, patients with *KCNJ11* mutations have potential to offer new insights into neurodevelopmental disorders. Clearly neurodevelopmental processes and the disorders associated with them are highly complex and are influenced by many factors including genetics, environment, socioeconomic context, physical co-morbidities, and clinical and educational interventions. Single gene causes of neurodevelopmental disorders are rare e.g. they account for only ~1% of

autism (49). However, monogenic disease again offers a unique platform for examining the role(s) of specific proteins in biological pathways, in this case those involved in neurodevelopment. Studying patients with *KCNJ11* neonatal diabetes allows investigation of the potential role(s) of K_{ATP} channels in the CNS and the therapeutic window of time for targeting these pathways with drug therapies. This may assist our understanding of developmental processes in the CNS more broadly as well as in the context of K_{ATP} channel-related syndromic diabetes. Importantly, in clinical practice it may assist in driving a precision medicine approach to developmental disorders.

Research collaborations and new study cohorts of the future

This research has facilitated successful collaboration with other centres internationally, which is of particular importance given the rarity of *KCNJ11* neonatal diabetes. These collaborations will be crucial in the future as we endeavour to address the large number of additional research questions in the field, many of which have arisen as a result of our studies. As discussed above, it is likely that ascertainment of existing cases and prompt identification of new cases will continue to increase in the future, particularly if neonatal diabetes is adopted into the newborn screening programme (43). This will give rise to larger research cohorts facilitating replication of existing findings as well as additional analyses that have not yet been possible given the small number of individuals available to study. An example of this would be using mendelian randomisation to investigate the impact of CYP450 genotype on glycaemic and CNS response to sulphonylureas in individuals with *KCNJ11* mutations. Furthermore, the number of monogenic pregnancies that can be followed up will rise over time. Technological advances such as cffDNA that make it possible to

genetically diagnose affected fetuses in utero will provide a valuable opportunity to further decipher the roles of insulin and K_{ATP} channels during early development, as well as the impact of sulphonylurea treatment of affected mothers (and by default their unborn children) in pregnancy.

Final conclusion

It has been an enormous privilege to have worked with patients with *KCNJ11* neonatal diabetes during the course of my research. The studies in this thesis have offered novel insights into the treatment response to sulphonylureas in affected individuals as well as the CNS phenotype, and have had a significant impact on clinical care. Importantly, the work has also provided a foundation for future research in the field, which will have utility in further refining care pathways specific to neonatal diabetes and more broadly in relation to understanding precision medicine and biological pathways related to K_{ATP} channels.

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APPENDIX 1

APPENDIX 1

CONTENTS

Long-term surveillance of Patients with Permanent Neonatal Diabetes

Please return by email to A.T.Hattersley@exeter.ac.uk or fax to +44 1392 408388 or post to Professor Andrew Hattersley, University of Exeter Medical School, Level 3 RILD Building, Barrack Road, Exeter EX25DW.
Thank you very much for your help.

Patient details:

Name		Gender	Female
MODY number		Ethnicity	
Date of birth		Gene (mutation)	

Any other current medication? If yes please give names and doses.

Date Sulphonylurea started:

Date insulin stopped

Before Transfer - *Please give 1-2 values prior to transfer from insulin.*

Date visit	Insulin dose (U/Day)	HbA1c (%)	Weight (kg)	Height (cm)	Other medication (please give names and doses)

Number of episodes of severe hypoglycaemia* in 12 months prior to transfer

Number of episodes of ketoacidosis in 12 months prior to transfer

Severe Hypoglycaemia* = *Semi-conscious / unconscious or in coma ± convulsions or needs assistance e.g. parenteral therapy (glucagon or IV glucose).* *Ketoacidosis= Needs admission to hospital for hyperglycaemia.

After Transfer - *Please give one set of values per year as close to anniversary of transfer date as possible.*

Year	Date of visit	SU name	SU dose (mg/day)	SU formula eg. Tablets, suspension	HbA1c/ % (mmol/ mol)	Weight (kg)	Height (cm)	Insulin required (U/day)	Severe hypos (number)	keto-acidosis (number)
6 mo										
1										
2										
3										
4										
5				.						
6				.						
7										
8				.						
9				.						
10										
11										
12										

Side effects whilst on SU treatment (YES/NO/NK**(not known)* **for each)

Gastrointestinal		Abnormal liver function	
Hypersensitivity		Abnormal kidney function	
Photosensitivity		Tooth discolouration	

If YES to any of above please give details including dates and action taken (e.g. dose reduction):	

Neurological features (YES/NO/NK *(not known)* ***for each)***

Developmental Delay		Muscle weakness	
Learning Difficulties		Epilepsy	
Sleep problems		Anxiety	
ADHD (attention deficit hyperactivity disorder)		Autism	
Other			

Did neurological features change with sulphonylurea treatment? If yes please give details:	

Cardiovascular risk factors (Please enter value for each from most recent assessment)

Date reviewed			
Blood Pressure (mmHg)		HDL Cholesterol (mmol/L)	
Triglycerides (mmol/L)		LDL Cholesterol (mmol/L)	
Total Cholesterol (mmol/L)			

Puberty and Development (Please enter value from most recent assessment)

Date reviewed			
Present pubertal stage		Age of Tanner 5 if appropriate (years, months)	
Age of Tanner 1 if appropriate (years, months)		Age of menarche (years, months)	

Other co-morbidity (YES/NO/NK (not known) for each)

Coeliac disease		Hypothyroidism	
Other			

Please give details including date of diagnosis/diagnostic test/treatment*:*	

Any other comments:	

Persistent microalbuminuria (3 consecutive measurements)		MI/peripheral vascular disease	
Proteinuria / Raised creatinine		Stroke	
Retinopathy		Neuropathy	
Other			

If YES to any of above please give details and dates e.g. if retinopathy state whether proliferative, non-proliferative or pre-proliferative and when first observed:	

Clinician name:	Email:	Date:

Thank you very much in advance for your help. Please don't hesitate to contact me if you need any assistance.

FoND Study Meal Compositions

High Carb Meal	Weight (g) or volume (ml)	Calories	Fat (g)	Protein (g)	Carbs (g)
White toast	2 x 40g = 80g	186.4	1.4	7.0	35.7
Jam	30g	77.7	0	0.1	19.1
Orange juice	250ml	107.5	0	2.0	22.3
TOTAL		371.6	1.4g	9.1g	77.1

Table 1. High carbohydrate meal composition.

High Protein Meal 1	Weight (g)	Calories	Fat (g)	Protein (g)	Carbs (g)
Low fat cheddar cheese (be good to yourself) 4 slices	96	272.6	16.3	28.5	3.0
Lean ham (Danepack), 4 slices	80	96.8	2.0	17.2	2.4
TOTAL		369.4	18.3	45.7	5.4

Table 2a. High protein meal composition with Danepak ham.

High Protein Meal 2	Weight (g)	Calories	Fat (g)	Protein (g)	Carbs (g)
Low fat cheddar cheese (be good to yourself) 4 slices	96g	272.6	16.3	28.5	3.0
Lean ham (Welly), 7.1 slices	91g	96.4	2.1	17.3	2.55
TOTAL		369.0	18.4	45.8	5.55

Table 2b. High protein meal with quantity of ham adjusted for different brand (Welly).

FoND Study Visit X

Baseline Data Collection Sheet

Patient Name	
D.O.B.	
Study ID	
Weight (kg)	
Height (cm)	
Any changes in health / medication since last visit? If so please give details.	
Time last food / drink taken	
Any additional comments	

Name of researcher collecting baseline data _____

Date _____

Patient Name; _____ Visit 1 / 2 / 3 (please circle)

FoND hypoglycaemia screening questions Time -5

	Absent						Severe
Neuroglycopenic symptoms							
difficulty speaking	1	2	3	4	5	6	7
confusion	1	2	3	4	5	6	7
dizziness	1	2	3	4	5	6	7
irritability	1	2	3	4	5	6	7
drowsiness	1	2	3	4	5	6	7
Autonomic symptoms							
sweating	1	2	3	4	5	6	7
anxiety	1	2	3	4	5	6	7
tremor	1	2	3	4	5	6	7
palpitations	1	2	3	4	5	6	7
feeling hot	1	2	3	4	5	6	7

TOTALS Neuroglycopenic ____

Auntonomic ____

Overall_____

Patient Name; _____ ***Visit 1 / 2 (please circle)***

FoND hypoglycaemia screening questions ***Time 30***

	Absent						Severe
Neuroglycopenic symptoms							
difficulty speaking	1	2	3	4	5	6	7
confusion	1	2	3	4	5	6	7
dizziness	1	2	3	4	5	6	7
irritability	1	2	3	4	5	6	7
drowsiness	1	2	3	4	5	6	7
Autonomic symptoms							
sweating	1	2	3	4	5	6	7
anxiety	1	2	3	4	5	6	7
tremor	1	2	3	4	5	6	7
palpitations	1	2	3	4	5	6	7
feeling hot	1	2	3	4	5	6	7

TOTALS Neuroglycopenic ____

Auntonomic ____

Overall_____

Patient Name; _____ ***Visit 1 / 2 (please circle)***

FoND hypoglycaemia screening questions ***Time 60***

	Absent						Severe
Neuroglycopenic symptoms							
difficulty speaking	1	2	3	4	5	6	7
confusion	1	2	3	4	5	6	7
dizziness	1	2	3	4	5	6	7
irritability	1	2	3	4	5	6	7
drowsiness	1	2	3	4	5	6	7
Autonomic symptoms							
sweating	1	2	3	4	5	6	7
anxiety	1	2	3	4	5	6	7
tremor	1	2	3	4	5	6	7
palpitations	1	2	3	4	5	6	7
feeling hot	1	2	3	4	5	6	7

TOTALS Neuroglycopenic ____

Auntonomic ____

Overall_____

Patient Name; _____ ***Visit 1 / 2 (please circle)***

FoND hypoglycaemia screening questions ***Time 90***

	Absent						Severe
Neuroglycopenic symptoms							
difficulty speaking	1	2	3	4	5	6	7
confusion	1	2	3	4	5	6	7
dizziness	1	2	3	4	5	6	7
irritability	1	2	3	4	5	6	7
drowsiness	1	2	3	4	5	6	7
Autonomic symptoms							
sweating	1	2	3	4	5	6	7
anxiety	1	2	3	4	5	6	7
tremor	1	2	3	4	5	6	7
palpitations	1	2	3	4	5	6	7
feeling hot	1	2	3	4	5	6	7

TOTALS Neuroglycopenic ____

Auntonomic ____

Overall_____

Patient Name; _____ ***Visit 1 / 2 (please circle)***

FoND hypoglycaemia screening questions ***Time 120***

	Absent						Severe
Neuroglycopenic symptoms							
difficulty speaking	1	2	3	4	5	6	7
confusion	1	2	3	4	5	6	7
dizziness	1	2	3	4	5	6	7
irritability	1	2	3	4	5	6	7
drowsiness	1	2	3	4	5	6	7
Autonomic symptoms							
sweating	1	2	3	4	5	6	7
anxiety	1	2	3	4	5	6	7
tremor	1	2	3	4	5	6	7
palpitations	1	2	3	4	5	6	7
feeling hot	1	2	3	4	5	6	7

TOTALS Neuroglycopenic ____

Auntonomic ____

Overall_____

Patient Name; _____ ***Visit 1 / 2 (please circle)***

FoND hypoglycaemia screening questions ***Time 150***

	Absent						Severe
Neuroglycopenic symptoms							
difficulty speaking	1	2	3	4	5	6	7
confusion	1	2	3	4	5	6	7
dizziness	1	2	3	4	5	6	7
irritability	1	2	3	4	5	6	7
drowsiness	1	2	3	4	5	6	7
Autonomic symptoms							
sweating	1	2	3	4	5	6	7
anxiety	1	2	3	4	5	6	7
tremor	1	2	3	4	5	6	7
palpitations	1	2	3	4	5	6	7
feeling hot	1	2	3	4	5	6	7

TOTALS Neuroglycopenic ____

Auntonomic ____

Overall_____

Patient Name; _____ ***Visit 1 / 2 (please circle)***

FoND hypoglycaemia screening questions ***Time 180***

	Absent						Severe
Neuroglycopenic symptoms							
difficulty speaking	1	2	3	4	5	6	7
confusion	1	2	3	4	5	6	7
dizziness	1	2	3	4	5	6	7
irritability	1	2	3	4	5	6	7
drowsiness	1	2	3	4	5	6	7
Autonomic symptoms							
sweating	1	2	3	4	5	6	7
anxiety	1	2	3	4	5	6	7
tremor	1	2	3	4	5	6	7
palpitations	1	2	3	4	5	6	7
feeling hot	1	2	3	4	5	6	7

TOTALS Neuroglycopenic ____

Auntonomic ____

Overall_____

Patient Name; _____ ***Visit 1 / 2 (please circle)***

FoND hypoglycaemia screening questions ***Time 210***

	Absent						Severe
Neuroglycopenic symptoms							
difficulty speaking	1	2	3	4	5	6	7
confusion	1	2	3	4	5	6	7
dizziness	1	2	3	4	5	6	7
irritability	1	2	3	4	5	6	7
drowsiness	1	2	3	4	5	6	7
Autonomic symptoms							
sweating	1	2	3	4	5	6	7
anxiety	1	2	3	4	5	6	7
tremor	1	2	3	4	5	6	7
palpitations	1	2	3	4	5	6	7
feeling hot	1	2	3	4	5	6	7

TOTALS Neuroglycopenic ____

Auntonomic ____

Overall_____

Patient Name; _____ ***Visit 1 / 2 (please circle)***

FoND hypoglycaemia screening questions ***Time 240***

	Absent						Severe
Neuroglycopenic symptoms							
difficulty speaking	1	2	3	4	5	6	7
confusion	1	2	3	4	5	6	7
dizziness	1	2	3	4	5	6	7
irritability	1	2	3	4	5	6	7
drowsiness	1	2	3	4	5	6	7
Autonomic symptoms							
sweating	1	2	3	4	5	6	7
anxiety	1	2	3	4	5	6	7
tremor	1	2	3	4	5	6	7
palpitations	1	2	3	4	5	6	7
feeling hot	1	2	3	4	5	6	7

TOTALS Neuroglycopenic ____

Auntonomic ____

Overall_____

STANDARD OPERATING PROCEDURE (SOP)

FoND Study

BLOOD SAMPLE HANDLING, TRANSPORT AND ANALYSIS.

VERSION NUMBER:	2.0
EFFECTIVE DATE:	15th July 2016
REVIEW DATE:	
AUTHOR:	Pam Bowman, University of Exeter Medical School Tim McDonald, Central laboratory, Exeter
APPROVED BY (1):	Steve Spaull, CRF, University of Exeter Medical School
DATE APPROVED:	15/07/16

Procedure for Visits 1 & 2

Blood Samples Handling, Transport & Analysis

To be used in conjuction with Protocol: CRF 227 FoND Study Version 1 29/04/16

1. Purpose.
The purpose of this SOP is to outline the procedure for the blood sample handling, transport and analysis in the FIND Study (Impact of Food on Insulin Secretion in Neonatal Diabetes).

2. Applicability.

This SOP applies to; study personnel involved in the blood sampling of patients.

3. Procedure.
Patients will fast from 10pm the night before the visits. They can take water, but no other drinks. They will be asked, if applicable, to avoid excessive alcohol and exercise for 48 hrs prior to this appointment.

Patients will attend between 0700 and 1000am. Following the (optional) application of topical anaesthetic cream, a standard gauge cannula will be placed into a forearm vein for blood sampling.

Blood Samples

Ensure you have the correct sample collection kit (and barcode set ***FN-XX-XXXX***) and correct blood tube types (see table 1). Label appropriate labels or barcodes on the blood collection tubes. Collect all blood tubes as indicated in table 1. This should total 2 primary blood tubes (serum and BD-P800). Ensure all primary blood tubes are mixed at collection by 10x gentle inversion.

Flush cannula with 3-5ml sterile normal saline if there is 30 minutes or more until the next sample is due. If cannula has been flushed, before next blood sample withdraw 0.5ml of blood and discard prior to filling tubes.

At each time point (-5, 0, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240 mins) collect 3ml blood in a 5ml syringe. Decant 2ml into 1 BDP-800 tube using a blunt fill needle. Eye protection may be worn when decanting blood to avoid blood splash injury. Use the remining blood in the syringe to measure blood glucose on the YSI and record this reading. At each time point also use an adaptor to fill one serum tube (4.9ml). Time point 0 is immediately before eating the meal and time point 15 mins is 15 minutes after starting to eat the meal. Participants will only be allowed 15 minutes to eat the meal therefore will have finished by the time the 15 minute sample is taken.

Centrifugation

It is important that all baseline blood samples are centrifuged (as appropriate/ necessary) and processed as soon as possible after sample collection.

Centrifuge the BDP-800 tubes indicated in table 1 at room temperature for 10 minutes at 1300G. Centrifuge the serum tubes at room temperature for 10 minutes at 2500G. Once centrifuged, transfer separated plasma promptly into labelled storage tubes, or 2D barcode tubes and transfer into appropriate storage racks. See table 1.

Process:	Source primary tubes →	Stick labels / barcodes onto the primary tubes →	Centrifuge if necessary →	Stick labels / barcodes onto the aliquot tubes →	Fill aliquot tubes →	Check all labels in barcode set are used →	Actions for primary tubes

Primary tube type	Primary tube label / barcode	Centrifuge primary sample	Aliquot tube label / barcode	Large aliquots	2d barcode tube aliquots		Directions for primary tube
Serum (4.9ml for adult participants or 2.7ml for paediatric participants)	label	Yes	1 x barcode 4x small labels	500uL for insulin, glucose, paracetamol	1x Elkay into insulin box x4 2D aliquots into storage box at -80C (X2 2D aliquots for children)		Dispose after having made aliquots
BDP-800 (2ml) – collected in syringe and decanted (same size for adults and children)	label	Yes	2 x small label	none	x1 into GLP-1 box at -80C x1 into GIP box at -80C		Dispose after having made aliquots

Table 1: Sample handling procedure for baseline bloods

Barcodes

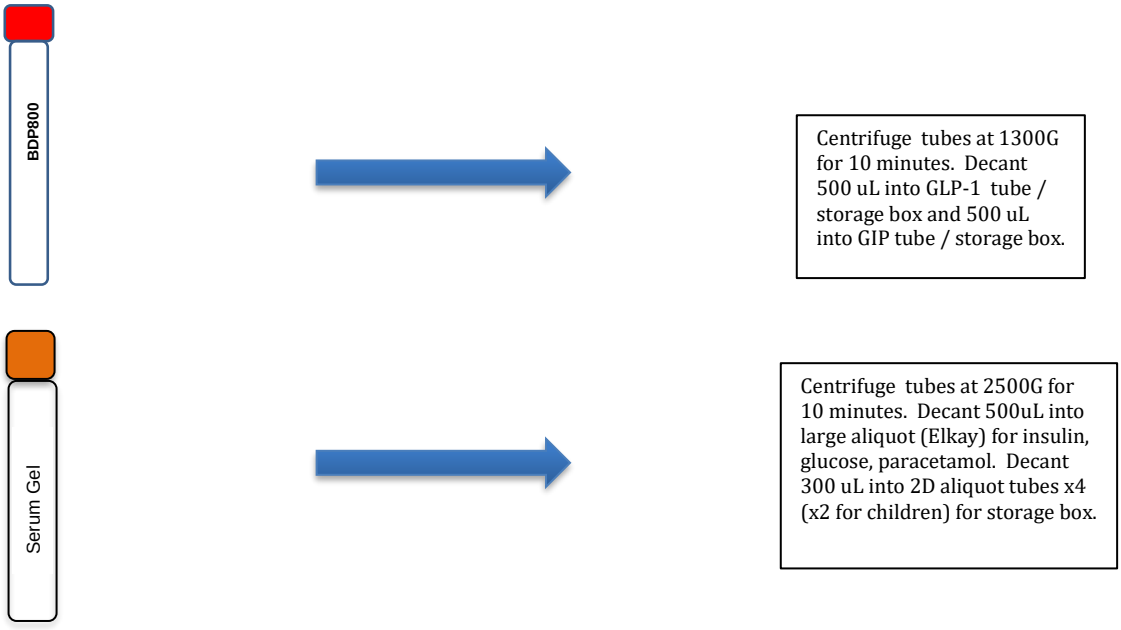
Samples should be identifiable by the bar code. Use only the bar codes provided for the study (FN-XX-xxxx).

Transport of samples to laboratory

Sample shipments can be organized at any time (weekday collection and delivery only). It is preferable to transfer full sample storage boxes only. Where possible, samples should be batched together for convenience and economy. Samples must be shipped on dry ice.

A summary of the sampling tubes to be used and the processing requirements is provided in Figure 1. The tube lid colour may vary according to the manufacturer supplying the tubes.

Figure 1: Summary of Visits 1 & 2 sample collection



FoND Study Flowchart

Patient Name_____ Date_____

Meal A / B /C (please circle) Weight_____ Height_____

Time (mins)	Blood sample	YSI	YSI Value	Hypo questions	Other / comments
INSERT CANNULA.					
-5	**YES**	**YES**	_____	**YES**	
0	**YES**	**YES**	_____	NO	Start Breakfast
15	**YES**	**YES**	_____	**NO**	Finish Breakfast
30	**YES**	**YES**	_____	**YES**	
45	**YES**	**YES**	_____	**NO**	
60	**YES**	**YES**	_____	**YES**	Flush cannula
90	**YES**	**YES**	_____	**YES**	Flush Cannula
120	**YES**	**YES**	_____	**YES**	Flush cannula
150	**YES**	**YES**	_____	**YES**	Flush cannula
180	**YES**	**YES**	_____	**YES**	Flush cannula
210	**YES**	**YES**	_____	**YES**	Flush cannula
240	**YES**	**YES**	_____	**YES**	
END OF STUDY. REMOVE CANNULA. PATIENT HAS LUNCH.					

***Name of researcher collecting samples*_____**

Please tick off each blood sample / questionnaire on flowchart as it is taken / completed and write glucose value from YSI in space provided at each time point. Please note any other events in the comments section, state time and give details e.g. participant went to toilet, extra flush used etc.

Blood sampling

- At each time point collect
    - One standard (4.9ml) serum tube
    - 3ml of blood in a 5ml syringe
        - Decant 2ml into standard BDP-800 tube (only fill as much as vacuum draws into tube through needle), mix by gentle inversion
        - Use remaining blood to get BG reading from YSI
- For paediatric participants collect blood in syringe for BDP-800 bottle and YSI and one small (2.9ml) serum tube.
- Flush cannula with 3-5ml normal saline if there is 30 minutes or longer until the next blood sample is due, or if cannula stops working and needs a flush at any other point (please document this on flowchart if it occurs).
    - Withdraw and discard 0.5ml of blood before filling sample tubes if cannula has been flushed at previous time point.
- Samples will be collected from bedside and transferred to technicians for processing.
- Please ensure all samples are appropriately labelled with printed label prior to collection.

Questionnaires

- Complete a separate questionnaire at -5 minutes, 30 minutes then every half an hour to screen participant for symptoms of hypoglycaemia.

Heights and Weights

- Please measure the height and weight of the study participant during one of the half hour intervals between blood tests (or at the end of the study) and add these values to the flowchart in the spaces provided.

Meals

- Meals will be either high protein or high carbohydrate – order will be allocated at random.
- Encourage patient to eat all food in 15 minutes. When 15 minutes finished and / or patient has finished eating, ensure leftovers are kept and returned to kitchen for weighing.

Management of severe hypoglycaemia

We expect that blood glucose readings may drop lower than normal. No clinical intervention is needed **unless**

1. Blood glucose <3mmol/l
   AND
2. Neurological symptoms present
    a. Confusion
    b. Drowsiness / reduced consciousness
    c. Seizures

If intervention is needed please use attached flowchart to manage hypoglycaemia. Participation in study will end at point of intervention, if treatment is required following doctor review.

Discharge from CRF

Cannula can be removed 10 minutes after the last blood sample provided there are no clinical concerns. Patients can go home after lunch (which we will provide) if they feel well and there are no clinical concerns.

9. What will happen to the samples I provide?

You will be asked for blood samples at each visit . Your samples will be stored using a code, without your name or other personal details (anonymised) and stored for the specialist tests. Access to samples or information related to samples is restricted to members of the research team only. You will be given the option to donate any spare samples available at the end of the study and the anonymised research data related to your samples to an approved tissue bank (Peninsula Research Bank) for use in future biomedical research. Your identity will remain anonymous to researchers using these samples/data.

10. Will I be told about the results of the study?

We expect the project to take 1-2 years to complete. At the end of the project, a copy of the study findings will be made available to participants. We will write up the results for publication. When published, copies of any publications will be available to be viewed and downloaded from our diabetesgenes.org website. You will not be identifiable in these publications.

11. Will I be reimbursed for any expenses during the study?

Yes, all reasonable expenses incurred by your participation in the study will be reimbursed.

12. Do I have to take part in this study?

No, participation is entirely voluntary. It is up to you to decide to join the study. If you agree to take part, we will ask you to sign a consent form, and you will be given the opportunity to discuss any questions with a member of the research team. You are also free to withdraw from the study at any point without giving reason, and this would not affect your clinical care. If you do decide to take part, you will be asked to consent to the following statements with a member of the research team present:

- I confirm that I have been provided with an information sheet for the above study. I have had opportunity to consider the information, ask questions and have had these questions answered satisfactorily.
- I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.
- I am happy to have an intravenous cannula inserted and multiple blood samples taken.
- I am happy to take a one-off standard dose of paracetamol/calpol before each meal.
- I give permission for NHS staff within the Exeter Clinical Research Facility to access my medical records.
- I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.
- I agree to take part in this study.

OPTIONAL STATEMENTS

- I am happy to gift any spare samples available at the end of the study and the research data relating to this study to the Peninsula Research Bank so that they can be used in future research studies providing that my identifying information is not shared with other researchers or organisations.
- I give permission for my contact details to be kept on a secure database to be contacted about other research in the future.

Finally...

Thank you for taking the time to read this information. Before you make a decision about participating in this study, you may want to discuss the project with your GP or family members. If you decide that you wish to take part, please contact Dr Pamela Bowman (Principle Investigator) on 01392 408325 or Dr Bea Knight (Research Nurse) on 01392 408172.

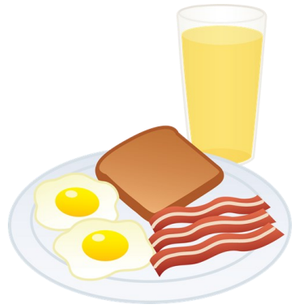
University of Exeter

Royal Devon and Exeter NHS Foundation Trust

The FoND study

Assessing the effect of **Fo**od composition on postprandial insulin secretion in KCNJ11 **N**eonatal **D**iabetes

Can you help us to see if different amounts of protein and sugar in food can affect how much insulin is released from the pancreas in people with neonatal diabetes?



What participation would involve:

- 2 visits to our research facility (2-3 days apart) each lasting approx. 5 hours.
- Donation of blood samples before and after a meal.
- On one visit the meal will be a high protein/low carbohydrate mix and on the other visit it will be a low protein/high carbohydrate mix (e.g. cheese and ham or eggs / meat alternative for one meal and orange juice and white bread toast with jam for the other meal).
- Taking a one-off dose of sugar free paracetamol with each meal.

Contents:

- **Before you decide whether to take part, it is important to understand why the research is being done and what it will involve.**
- **Please take the time to read the following information carefully.**
- **You are free to decide if you want to take part in this research study.**
- **You can decide to stop taking part in the study at any time without giving a reason.**
- **Please ask us if anything is not clear or if you would like more information.**

How to contact us:

If you have any questions about this study or would like to be involved, please contact Dr. Pam Bowman (Principle Investigator) on 01392 408325 or Dr Bea Knight (Research Nurse) on 01392 408172.

IRAS Project ID 205255 **FoND/Adults/PIS/V3/120616**

1. Why are we doing this research?

People with neonatal diabetes who switch from insulin to sulphonylurea treatment have better blood glucose control, including fewer episodes of low blood sugars (hypoglycaemia). If hypoglycaemia does occur it is usually mild and self-limiting and may be related to the type of food being eaten. We would like to explore this idea in more detail. We can do this by looking at the effect of eating different combinations of protein and carbohydrate on the amount of insulin, glucose and incretin hormone in the blood afterwards. By also comparing the results from people without diabetes and those with Type 2 diabetes who are also treated with sulphonylureas (our control groups) we hope to be able to understand how sulphonylureas work in different types of diabetes and improve the advice we give to patients about their diet and reducing the risk of hypoglycaemia.

2. Am I eligible to take part?

You will be able to take part if you have: neonatal diabetes treated with sulphonylureas, Type 2 diabetes treated with sulphonylureas or do not have diabetes. You will not be able to take part if you have any known allergies to paracetamol.

3. Are there any risks in taking part?

We do not anticipate there being any risks to your health by taking part in the study.

- As some people with neonatal diabetes have reported mild hypoglycaemia (blood sugar going low) after food containing little carbohydrate you will be closely monitored during your visits to ensure this does not cause you any problems.
- Blood sampling can be uncomfortable and may cause bruising but this will be carried out by researchers who have had lots of practice in these procedures to reduce these risks.
- If we happen to discover anything unexpected, we will inform your GP or the relevant healthcare professional. Entering into the study is unlikely to affect your current treatment.

4. What if I don't like or can't take the food options?

We will discuss any special dietary needs you may have and try to accommodate these and provide alternatives to our standard offerings.

5. What are the benefits of taking part?

There may be no direct benefits to you from taking part in this study, but your contribution will help us to understand more about the effects of different foods on insulin release and the role of other hormones in this process. This will be important for providing advice about diet and hypoglycaemia risk to people with neonatal diabetes who are on sulphonylurea treatment.

6. Will my participation be confidential?

Any information you provide will be held in the strictest confidence. If you take part in the study, your information will be coded. All information that is collected about you will be held on a password-protected computer. Access to data will be available to the research team only. Anonymous data will be stored for five years, or indefinitely if you give permission for the data to be transferred to the Peninsula Research Bank (PRB). This data may be made available for future research projects.

7. What will I need to do if I take part?

The study involves 2 appointments. Details of what will happen at each visit and how you will be asked to prepare are shown below.

Research Visit 1 (5 hours)

- Avoid excessive exercise or alcohol for 48 hours before taking part.
- Arrive at the Exeter Clinical Research Facility (CRF) having fasted overnight (continue to take your regular medicines if appropriate but nothing containing paracetamol for 24 hours before taking part).
- Provide written consent for participation.
- Baseline information obtained: height and weight measured and questions asked about your health and any current treatments.
- A needle will be used to introduce a small plastic tube (cannula) into one of the veins in your hand or arm. The needle is removed immediately leaving only the soft cannula in place. All blood samples will then be taken easily and painlessly through this tube which will be left in your hand or arm throughout your study visit.
- Blood samples will be taken to measure levels of glucose and the hormones involved in controlling blood sugar levels.
- You will then be given a breakfast to eat which will either be high protein/low carbohydrate or low protein/high carbohydrate (you will not be told which it is).
- After the meal. you will have more blood samples taken at regular intervals over 4 hours, to measure the same things that were measured in the first blood test. This will allow us to see how the levels change over time.
- You will also be asked to take some sugar free paracetamol with the meal, and paracetamol levels will be measured with each blood test to tell us how quickly the stomach is emptying.
- Your will also regularly be asked some questions to see how you are feeling. If you feel unwell at any point, help will be immediately available from a member of the research team.

Minimum 2-day interval

Research Visit 2 (5 hours)

- The procedure will be the same as visit 1, but at this visit you will be given a breakfast of the opposite combination to the one eaten at visit 1.
- This will then complete the sample collection part of the study.

8. Who is organising this study?

This project is being run at the NIHR Exeter Clinical Research Facility (at the Royal Devon and Exeter Hospital, Wonford, site) with the University of Exeter. It has been reviewed by a Research Ethics Committee (REC) prior to starting (South West—Cornwall & Plymouth REC). The Patient Advice and Liaison Service (PALS) can provide independent advice about research participation, and can provide assistance if you have any concerns: (tel: 01392 402093 or email: rde-tr.PAL@nhs.net)

10. Will I be told about the results of the study?

We expect the project to take 1-2 years to complete, including these additional tests. At the end of the project, a copy of the study findings will be made available to participants. We will write up the results for publication. When published, copies of any publications will be available to be viewed and downloaded from our diabetesgenes.org website. You will not be identifiable in these publications.

11. Do I have to take part in these extra tests?

No, participation is entirely voluntary. It is up to you to decide whether you would like to take part in one or both of the additional tests. If you agree to take part, we will ask you to sign a consent form, and you will be given the opportunity to discuss any questions with a member of the research team. You are also free to withdraw from the study at any point without giving reason, and this would not affect your clinical care. If you do decide to take part, you will be asked to consent to the following statements with a member of the research team present:

- I confirm that I have been provided with an information sheet for the additional tests for the FoND study. I have had opportunity to consider the information, ask questions and have had these questions answered satisfactorily.
- I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.
- I am happy to have an intravenous cannula inserted and multiple blood samples taken.
- I am happy to take a one-off standard dose of paracetamol at each of the visits.
- I give permission for NHS staff within the Exeter Clinical Research Facility to access my medical records.
- I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.
- I agree to take part in visit 3 involving blood sampling without a breakfast.
- I agree to take part in visit 4 involving blood sampling before and after a breakfast.

OPTIONAL STATEMENTS

- I am happy to gift any spare samples available at the end of the study and the research data relating to this study to the Peninsula Research Bank so that they can be used in future research studies providing that my identifying information is not shared with other researchers or organisations.
- I give permission for my contact details to be kept on a secure database to be contacted about other research in the future.

Finally...

Thank you for taking the time to read this information. Before you make a decision about participating in these additional parts of the FoND study, you may want to discuss the project with your GP or family members. If you decide that you wish to take part, please contact Dr Pamela Bowman (Principle Investigator) on 01392 408325 or Dr Bea Knight (Research Nurse) on 01392 408172.

University of Exeter

Royal Devon and Exeter NHS Foundation Trust

The FoND study

Optional Additional Tests

Assessing the effect of **F**ood composition on postprandial insulin secretion in KCNJ11 **N**eonatal **D**iabetes

Can you help us to understand even more about the glucose and insulin response to food in neonatal diabetes treated with sulphonylureas?

What participation would involve:

- 1 or 2 additional visits to our research facility (2-3 days apart) each lasting approx. 5 hours.
- Donation of blood samples.
- On one visit there would be no meal and on the other there would be a mixture of protein, fat and carbohydrate (e.g. ham, cheese, bread and orange juice).
- Taking a one-off dose of sugar free paracetamol at each visit.

Contents:

- **Before you decide whether to take part, it is important to understand why the extra piece of research is being done and what it will involve.**
- **Please take the time to read the following information carefully.**
- **You are free to decide if you want to take part in the additional tests for this study.**
- **You can decide to stop taking part in the study at any time without giving a reason.**
- **Please ask us if anything is not clear or if you would like more information.**

How to contact us:

If you have any questions about this study or would like to be involved, please contact Dr. Pam Bowman (Principle Investigator) on 01392 408325 or Dr Bea Knight (Research Nurse) on 01392 408172.

IRAS Project ID 205255 **FoNDExtra/Adults/PIS/V1/200916**

1. Why are we doing this extra piece of research?

You have already helped us to look at the effect of eating different combinations of protein and carbohydrate on the amount of insulin, glucose and incretin hormone in the blood afterwards. We would now like to do some additional tests to help understand these responses even better. We would like to know if the sulphonylurea tablet on its own has any effect on insulin and glucose levels, in the absence of a meal. We would also like to know if a mixture of protein and carbohydrate causes a different response to the individual protein and carbohydrate meals. By comparing the results in people with sulphonylurea-treated neonatal diabetes to people without diabetes and those with sulphonylurea-treated Type 2 diabetes (our control groups) we hope to be able to better understand how sulphonylureas work in different types of diabetes. We also hope to improve the advice we give to patients about their diet and reducing the risk of hypoglycaemia.

2. Am I eligible to take part?

You will be able to take part if you have previously participated in the FoND study.

3. Are there any risks in taking part?

We do not anticipate there being any risks to your health by taking part in the study.

- As one of the additional tests would involve taking your usual sulphonylurea medication (if applicable) in the absence of food, your blood glucose will be closely monitored during your visit(s) to ensure this does not cause you any problems.
- Blood sampling can be uncomfortable and may cause bruising but this will be carried out by researchers who have had lots of practice in these procedures to reduce these risks.
- If we happen to discover anything unexpected, we will inform your GP or the relevant healthcare professional. Participating in these extra tests is unlikely to affect your current treatment.

4. What are the benefits of taking part?

There may be no direct benefits to you from taking part in these additional tests, but your contribution will help us to understand more about the effects of different foods and sulphonylurea medication on insulin release and the role of other hormones in this process, which could help the clinical care of people with neonatal diabetes.

5. Will my participation be confidential?

As before, any information you provide will be held in the strictest confidence. If you take part in the extra tests, your information will be coded. All information that is collected about you will be held on a password-protected computer. Access to data will be available to the research team only. Anonymous data will be stored for five years, or indefinitely if you give permission for the data to be transferred to the Peninsula Research Bank (PRB). This data may be made available for future research projects.

6. Who is organising this study?

This project is being run at the NIHR Exeter Clinical Research Facility (at the Royal Devon and Exeter Hospital, Wonford, site) with the University of Exeter. It has been reviewed by a Research Ethics Committee (REC) prior to starting (South West—Cornwall & Plymouth REC). The Patient Advice and Liaison Service (PALS) can provide independent advice about research participation, and can provide assistance if you have any concerns: (tel: 01392 402093 or email: rde-tr.PAL@nhs.net)

7. What will I need to do if I take part?

You have already completed 2 research visits. The extra tests are optional and would involves another 1 or 2 appointments. Details of what will happen at each visit and how you

Research Visit 3 (5 hours)

- Avoid excessive exercise or alcohol for 48 hours before taking part.
- Arrive at the Exeter Clinical Research Facility (CRF) having fasted overnight (continue to take your regular medicines if appropriate but nothing containing paracetamol for 24 hours before taking part).
- Provide written consent for participation.
- Baseline information obtained: height and weight measured and questions asked about your health and any current treatments.
- A needle will be used to introduce a small plastic tube (cannula) into one of the veins in your hand or arm. The needle is removed immediately leaving only the soft cannula in place. All blood samples will then be taken easily and painlessly through this tube which will be left in your hand or arm throughout your study visit.
- Blood samples will be taken to measure levels of glucose and the hormones involved in controlling blood sugar levels.
- You will not be given anything to eat but you can continue to drink water. You will have blood samples taken at regular intervals over 4 hours, to measure the same things that were measured in the first blood test. This will allow us to see how the levels change over time.
- You will also be asked to take some sugar free paracetamol, and paracetamol levels will be measured with each blood test to tell us how quickly the stomach is emptying.
- Your will regularly be asked some questions to see how you are feeling. If you feel unwell at any point, help will be immediately available from a member of the research team.

Minimum 2-day interval

Research Visit 4 (5 hours)

- The procedure will be the same as visit 3, but at this visit you will be given a breakfast which will be a mixture of protein, fat and carbohydrate. Blood samples will be taken before and after the meal in the same way as your previous breakfast tests.
- This will then complete the sample collection part of the study.

8. What will happen to the samples I provide?

You will be asked for blood samples at each visit . Your samples will be stored using a code, without your name or other personal details (anonymised) and stored for the specialist tests. Access to samples or information related to samples is restricted to members of the research team only. You will be given the option to donate any spare samples available at the end of the study and the anonymised research data related to your samples to an approved tissue bank (Peninsula Research Bank) for use in future biomedical research. Your identity will remain anonymous to researchers using these samples/data.

9. Will I be reimbursed for any expenses during the study?

Yes, all reasonable expenses incurred by your participation in the study will be reimbursed.

Assessing the effect of food composition on postprandial insulin secretion in KCNJ11 neonatal diabetes (*the FoND Study*)

Chief Investigator: Andrew Hattersley **REC No: 16/SW/0150**

Consent Form – Participants aged 16 and over

Please **initial** the box if you agree with the statement below;

1. I confirm that I have been provided with an information sheet for the above study. I have had opportunity to consider the information, ask questions and have had these questions answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. ☐

3. I am happy to have an intravenous cannula inserted and multiple blood samples taken. ☐

4. I am happy to take a one-off standard dose of paracetamol with each of the meals. ☐

5. I give permission for NHS staff within the Exeter Clinical Research Facility to access my medical records. ☐

6. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

7. I agree to take part in this study. ☐

OPTIONAL STATEMENTS

8. I am happy to gift any spare samples available at the end of the study and the research data relating to this study to the Peninsula Research Bank so that they can be used in future research studies providing that my identifying information is not shared with other researchers or organizations. ☐

9. I give permission for my contact details to be kept on a secure database to be contacted about other research in the future. ☐

Participant's Name: Date: Signature
(Please print)

Researcher's Name: Date: Signature
(Please print)

Once completed: one original to be kept by the participant, one original to be kept by researcher.

Assessing the effect of food composition on postprandial insulin secretion in KCNJ11 neonatal diabetes (*the FoND Study*) – optional additional tests

Chief Investigator: Andrew Hattersley **REC No: 16/SW/0150**

Consent Form – Participants aged 16 and over who have already participated in FoND Study visits 1&2

Please **initial** the box if you agree with the statement below;

1. I confirm that I have been provided with an information sheet for the additional tests for the FoND study. I have had an opportunity to consider the information, ask questions and have had these questions answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. ☐
3. I am happy to have an intravenous cannula inserted and multiple blood samples taken. ☐
4. I am happy to take a one-off standard dose of paracetamol at each of the visits. ☐
5. I give permission for NHS staff within the Exeter Clinical Research Facility to access my medical records. ☐
6. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
7. I agree to take part in visit 3 involving blood sampling without a breakfast. ☐
8. I agree to take part in visit 4 involving blood sampling before and after a breakfast. ☐

OPTIONAL STATEMENTS

9. I am happy to gift any spare samples available at the end of the study and the research data relating to this study to the Peninsula Research Bank so that they can be used in future research studies providing that my identifying information is not shared with other researchers or organizations. ☐
10. I give permission for my contact details to be kept on a secure database to be contacted about other research in the future. ☐

Participant's Name: Date: Signature
(Please print)

Researcher's Name: Date: Signature
(Please print)

Once completed: one original to be kept by the participant, one original to be kept by researcher.

The Development and Well-Being Assessment

Parent Interview

Child's Surname:

Child's First Names:

Age:

Date of Birth:

Male / Female

Clinic/Study Number:

Date of Interview:

Person Interviewed:

Interviewer:

The first step is to administer the P4-16 Strengths and Difficulties Questionnaire (SDQ) and then score the front page, ringing the scores below. Subtract the prosocial score from the peer score – this will often be a negative number (because the prosocial score is usually higher than the peer score), but remember to show this as negative. Only positive scores of 2 or more are relevant to the DAWBA skip rule.

SDQ Emotion Score	**0**	**1**	**2**	**3 | | 4**		**5**	**6**	**7**	**8**	**9**	**10**
SDQ Hyperactivity Score	**0**	**1**	**2**	**3**	**4**	**5 | | 6**		**7**	**8**	**9**	**10**
SDQ Conduct Score	**0**	**1**	**2 | | 3**		**4**	**5**	**6**	**7**	**8**	**9**	**10**
SDQ Peer Score	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
SDQ Prosocial Score	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Peer minus Prosocial			**(positive score reflects peer score > prosocial score)** **(negative score reflects peer score < prosocial score)**								

Social Aptitudes Score	**(from Social Aptitudes Scale on page 2)**

Social Aptitudes Scale

How does [Name] compare with other children/people of his/her age in the following situations:

		A lot worse than average	A bit worse than average	About average	A bit better than average	A lot better than average
SAS1	Able to laugh around with others, for example accepting light-hearted teasing and responding appropriately.	0	1	2	3	4
SAS2	Easy to chat with, even if it isn't on a topic that specially interests him/her.	0	1	2	3	4
SAS3	Able to compromise and be flexible	0	1	2	3	4
SAS4	Finds the right thing to say or do in order to defuse a tense or embarrassing situation	0	1	2	3	4
SAS5	Graceful when s/he doesn't win or get his/her own way. A good loser.	0	1	2	3	4
SAS6	Other people feel at ease around him/her.	0	1	2	3	4
SAS7	By reading between the lines of what people say, s/he can work out what they are really thinking and feeling.	0	1	2	3	4
SAS8	After doing something wrong, s/he's able to say sorry and sort it out so that there are no hard feelings.	0	1	2	3	4
SAS9	Can take the lead without others feeling they are being bossed about.	0	1	2	3	4
SAS10	Aware of what is and isn't appropriate in different social situations.	0	1	2	3	4

Now score up the scale by adding all ten items, and enter the score on the first page.

Friendships Questionnaire

Fr1 What is [Name] like at making friends?

Finds it harder than average	About average	Finds it easier than average
0	1	2

Fr2 What is [Name] like at keeping the friends s/he has made?

Finds it harder than average	About average	Finds it easier than average
0	1	2

Fr3 At present, how many friends does s/he have that s/he fairly often spends time with, for example chatting, or doing things together, or going out as part of a group?

None	One	2-4	5-9	10+
0	1	2	3	4

↓ Next section

Fr4

Fr4 Do [Name] and his/her friends have interests in common?

No	A little	A lot
0	1	2

Fr5 Do [Name] and his/her friends take part in joint activities such as playing sport together, playing computer games together, or shopping together?

No	A little	A lot
0	1	2

Fr6 If [Name] were very stressed or had some secret worry, do you think s/he'd be able to talk about this with a friend and tell the friend how s/he was feeling?

No	Perhaps	Definitely
0	1	2

Fr 7 By and large, do you approve of his/her friends?

No	A little	A lot
0	1	2

Fr8 Are many of his/her friends the sorts of children/young people who often get into trouble for bad behaviour?

Not at all	A few are like that	Many are like that	All are like that
0	1	2	3

Development Section

R1 Thinking about his/her school work and about his/her ability to reason things out, is s/he about average for his/her age, ahead, or behind?

Ahead	Average	Behind
0	1	2

R3 → R2

R2 At present, roughly what sort of age level is s/he at in his/her school work and ability to reason things out? (*Optional*: For example like an average [*insert an age 2 years younger than the child's chronological age]* year old?)

If under 12 month level, code as '0'

[ ] years old

R3 Is his/her ability to use language – to say what s/he means and to understand what other people are saying – about average for his/her age, ahead or behind?

Ahead	Average	Behind
0	1	2

R6 → R4

R4 At present, roughly what sort of age level is s/he at in his/her use and understanding of language? (*Optional*: For example like an average [*insert an age 2 years younger than the child's chronological age]* year old?)

If under 12 month level, code as '0'

[ ] years old

R5 Can s/he get round difficulties in explaining what s/he wants to say by other means, for example by using gestures, signs, facial expressions or acting things out?

No	A little	A lot
0	1	2

R6 Going back to his/her first **3 years** of life, was there anything that seriously worried you or anyone else about:

		No	Yes
a)	the way his/her speech developed?	0	1
b)	how s/he got on with people?	0	1
c)	the way his/her pretend or make-believe play developed?	0	1
d)	any odd rituals or unusual habits that were *very* hard to interrupt?	0	1
e)	his/her ability to learn and do new things – things such as puzzles or helping get dressed?	0	1

Only ask if R6a, R6b, R6c,R6d orR6e = 'Yes'

R7 Have the things that seriously worried you or someone else now cleared up completely?

Cleared up completely	Some continuing problems
0	1

Skip rule for the rest of this section

Only continue if SAS score is 12 or less, if R7 = 'Some continuing problems', or if the SDQ peer problem score is 2 or more points higher than the prosocial score. Otherwise go to next section.

If R6a = 'Yes' continue with R8, else skip to R10

R8 Could s/he use any real words other than 'mama' or 'dada' before the age of 2 years?
(*Baby words such as 'bikkie' for 'biscuit' do count. Exclude other words for mother or father)*

No	Yes
0	1

R9 After using single words, children go on to join them up into phrases or short sentences like 'Go park see ducks' or 'Mama give biscuits'.

Did [Name] join words together into phrases or short sentences before the age of 3 years?
(*Do not count set phrases like 'Thank you' or 'Night night' that the child uses as just one word*)

No	Yes
0	1

R10 Toddlers often communicate through physical gestures such as waving goodbye, pointing to things, blowing a kiss, or bringing a finger to their mouth and saying Shh!

When s/he was a toddler, did [Name] use these sorts of gestures as much as other children of the same age?

About the same / More	A little less	A lot less
0	1	2

R11 Some children like playing nursery games like Ring a Ring a Roses, Round and Round the Garden, Peekaboo or Peepo.

Did [Name] ever like these games?

No	A little	A lot
0	1	2

R12 Young children often try to share their enjoyment or interests or achievements, for example by pointing out something that they think you will enjoy seeing or find interesting.

As a toddler and young child, did [Name] want to share his/her enjoyment, interests or achievements with other people?

No	A little	A lot
0	1	2

R13 Some children spend a lot of their play time repeating the same action over and over again, for example spinning the wheels on a toy car, turning taps or light switches on and off, or opening and shutting doors.

Has this ever been true of him/her?

No	A little	A lot
0	1	2

R14 Children are sometimes very interested in unusual aspects of toys or other things. For example, rather than playing with a toy, they may spend their time sniffing it, or running their fingers over its surface, or holding it to their face to feel any vibration that it makes.

Has this ever been true of him/her?

No	A little	A lot
0	1	2

R15 Make-believe play is important to some children. This can include pretend games with other children – games such as cops and robbers, or mummies and daddies. Even when they are by themselves, children may act out stories with dolls, action men or make-believe objects.

If aged under 11: As a preschool child and more recently, has [Name] taken part in make-believe play?

If aged 11 or more: Thinking about when s/he was younger (say between 5 and 10), did [Name] take part in make-believe play?

No	A little	A lot
0	1	2

R16 *If aged under 11*: At present, can [Name] make allowances according to who s/he is playing with? For example, not being too rough when playing with younger children, and not being too bossy when playing with older children.
If aged 11 or more: When s/he was younger (say around 10), could [Name] make allowances according to who s/he was playing with? For example, not being too rough when playing with younger children, and not being too bossy when playing things with older children.

No	A little	A lot
0	1	2

R17 When s/he's with other children/teenagers, does s/he have difficulty taking turns, sharing or co-operating?

No	A little	A lot
0	1	2

R18 Some children/teenagers enjoy putting a lot of time into collecting things, or get a lot of pleasure out of focusing on just one topic, such as sport, cars or a particular pop group. In everyday language, we often say that these children/teenagers are 'obsessed' by their interest, but this is not an unpleasant obsession – this is something they like and usually enjoy talking about.

Has [Name] had any long-lasting obsessions of this sort?

No	A little	A lot
0	1	2
		↓
R24		R19

R19 Obsessions may be about common or unusual topics. For example, it is fairly common for an 8 year old to be obsessed by dinosaurs, but it is unusual for an 8 year old to be obsessed by Victorian fireplaces, bar codes or street lamps.

Is or was the topic of his/her obsession unusual?

No	A little	A lot
0	1	2

R20 Does or did the obsession dominate his/her life?

No	A little	A lot
0	1	2

R21 Does or did it tend to dominate his/her conversation with other people?

No	A little	A lot
0	1	2

R22 Does or did it stop him/her doing other important things in his/her life, such as playing, studying or going out?

No	A little	A lot
0	1	2

R23 Please describe the obsession:

……..

……..

……..

R24 Is [Name] able to start conversations with other people?

No	A little	A lot
0	1	2

R25 If other people start conversations with him/her, can [Name] keep the conversation going?

No	A little	A lot
0	1	2

R26 Is [Name] genuinely interested in chatting with other people in order to hear what they have to say about their experiences and interests – even if those interests are different from his/her own interests?

No	A little	A lot
0	1	2

R27 Some children/teenagers have trouble adjusting their language to suit different social occasions. For example they may speak too casually to a teacher or too formally to other children.

Does [Name] change the way s/he speaks according to whether it is a formal or informal situation?

No	A little	A lot
0	1	2

R28 It is relatively easy to tell what some children/teenagers are feeling by observing their facial expressions, their tone of voice and their body language. It is harder to tell with other children/teenagers, particularly if you don't know them very well.

Do most people have difficulty knowing what [Name] is feeling by observing his/her face, body language or tone of voice?

No	A little	A lot
0	1	2

R29 The other way round, children/teenagers vary in their ability to know what other people are feeling. Some children/ teenagers are good at recognising subtle clues in body language, facial expressions, or tone of voice. For example, they can immediately tell when their mother is starting to get a little cross, or when another child/teenager is feeling a bit embarrassed. Other children/teenagers find this much harder.

Does [Name] have difficulty recognising the clues in other people's facial expressions, body language and tone of voice?

No	A little	A lot
0	1	2

R30 When we're talking with someone face-to-face, eye contact is very important. It generally makes us feel uneasy, or as if there's something wrong, if the other person makes too little eye contact, or too much, or makes it at the wrong time.

Has [Name] ever been through a phase of making too little or too much eye contact, or making it in the wrong sort of way?

No	Perhaps	Definitely
0	1	2

R31 Many young children go through a phase of repeating what someone has just said to them. For example, if you said, "We'll be going home in a few minutes", they might parrot back "We'll be going home in a few minutes". Or they might echo back the last word, "minutes", in your tone of voice. Some young people do this a lot.

Has [Name] ever echoed or parroted speech in this way?

No	A little	A lot
0	1	2

R32 Some children/teenagers ask the same questions over and over again. For example, "When are we going to the park?" or "What's for dinner?" or "Are we going swimming this weekend?" They keep repeating these questions even though they've already been told the answers many times. The questions that are repeated may not be exactly the same from week to week.

Has [Name] ever tended to ask repetitive questions?

No	A little	A lot
0	1	2

R33 Another way in which young people repeat themselves is by using the same catch-phrase or cliché over and over again. For example, almost every sentence may begin "If you want my opinion …" or "Logically speaking …" Occasionally the phrase is appropriate, but it is used far more than is really needed.

Has [Name] ever filled his/her speech with these fairly empty catch-phrases or clichés?

No	A little	A lot
0	1	2

R34 Some children enjoy routines and want things to be the same every day. For example, they may want to eat the same food off the same plate while sitting in the same chair every single day. Or there may be very fixed routines for dressing or undressing.

Has [Name] ever had strong or unusual routines that s/he has insisted on because s/he enjoyed doing it that way?

No	A little	A lot
0	1	2
	R36	→ R35

R35 Please describe the routines

……..

……..

……..

R36 Some children are easily upset by small changes in their routines. For example, they may be very upset by having to go to school a different way, by having to take a bath at a slightly different time, or by the furniture being moved around.

Has [Name] ever been easily upset by changes in routine?

No	A little	A lot
0	1	2

R37 Some preschool children go through a phase of flapping or waving their hands or arms up and down if they are excited or upset. Some continue doing this for years.

Since [Name] has been going to school, has s/he tended to flap his/her arms when excited or upset?

No	A little	A lot
0	1	2

R38 You have answered a lot of questions about his/her pattern of development – focusing particularly on language, play, routines and his/her ability to get along with other people.

Are you concerned at present about any of these aspects of his/her development?

No	A little	A lot
0	1	2
→ Next section	*tick Development on the check list in M1 (p.55) and continue with* R39	

R39 Thinking about the last 12 months, have difficulties in any of the areas that we have covered resulted in him/her becoming upset or distressed?

Not at all	A little	A medium amount	A great deal
0	1	2	3

R40 Have difficulties with language, routines, play, or social ability interfered with...

		Not at all	A little	A medium amount	A great deal
a)	how well s/he gets on with you and the rest of the family?	0	1	2	3
b)	making and keeping friends?	0	1	2	3
c)	learning or class work?	0	1	2	3
d)	playing, hobbies, sports or other leisure activities?	0	1	2	3

R41 Have these difficulties put a burden on you or the family as a whole?

Not at all	A little	A medium amount	A great deal
0	1	2	3

R42 Some children's development is unusual from birth onwards. With hindsight, their parents realise that development was never quite normal. That's not always the case, though. Sometimes parents are sure that development was completely normal for a while and then there was a relatively sudden change.

Which was true for him/her?

Always there to some extent	Sudden change
0	1
↓	↓
Next section	R43

R43 How old was [Name] when this change happened?

If during the first 12 months, code as '0'

[ ] years old

Section A Separation Anxiety

Most children are particularly attached to a few key adults, looking to them for security and comfort, and turning to them when upset or hurt.

A1	Is [Name] specially attached to the following adults?	No or Not Applicable	Yes
a)	Mother (biological or adoptive)	0	1
b)	Father (biological or adoptive)	0	1
c)	Another mother figure (stepmother, foster mother, father's partner)	0	1
d)	Another father figure (stepfather, foster father, mother's partner)	0	1
e)	One or more grandparents	0	1
f)	One or more other adult relatives (e.g. aunt, uncle, grown-up brother or sister)	0	1
g)	Childminder, nanny, au pair	0	1
h)	One or more teachers	0	1
i)	One or more other adult non-relatives (e.g. a family friend or neighbour)	0	1
j)	[ ] Not specially attached to any adult		

If A1j was ticked, ask A1k and A1l; otherwise continue with A2

	Is (Child) specially attached to the following children or young people?	No or Not Applicable	Yes
k)	One or more brothers, sisters or other young relatives	0	1
l)	One or more friends	0	1
m)	[ ] Not specially attached to anyone		

if A1m is ticked, then skip to section B. Otherwise continue:

A2 You've just told me who [Name] is specially attached to: *If you want, you can list all from A1a to A1i (or from A1k to A1l) that were answered 'Yes'.* From now on, I am going refer to these people as his/her 'attachment figures'

What I'd like to know next is how much [Name] worries about being separated from his/her attachment figures. Most children have some worries of this sort, but I'd like to know how [Name] compares with other children of his/her age. I am interested in how s/he is usually - not on the occasional 'off day'.

Overall, in the **last 4 weeks**, has s/he been particularly worried about being separated from his/her attachment figures?	No	Yes
	0	1

If A2 = Yes or if SDQ emotion score is ≥ 4 then continue. If neither, then skip to section B.

A3	Over the **last 4 weeks**, and compared with other children of the same age...	No more than others (or Not applicable)	A little more than others	A lot more than others
a)	has s/he worried either about something unpleasant happening to his/her attachment figures, or about losing them?	0	1	2
b)	has s/he worried unrealistically that s/he might be taken away from his/her attachment figures, e.g. by being kidnapped, taken to hospital or killed?	0	1	2
c)	Has s/he not wanted to go to school in case something nasty happened to his/her attachment figures while s/he was away at school? *(Do not include reluctance to go to school for other reasons e.g. fear of bullying or exams)*	0	1	2
d)	has s/he worried about sleeping alone?	0	1	2
e)	has s/he come out of his/her bedroom at night to check on, or to sleep near, his/her attachment figures?	0	1	2
f)	has s/he worried about sleeping in a strange place?	0	1	2
g)	*(Only ask if aged under11)* has s/he been afraid of being alone in a room at home without his/her attachment figures even if they are close by?	0	1	2
h)	*(Only ask if aged 11 or more)* has s/he been afraid of being alone at home if his/her attachment figures pop out for a moment?	0	1	2
i)	has s/he had repeated nightmares or bad dreams about being separated from his/her attachment figures?	0	1	2
j)	has s/he had headaches, stomach aches or felt sick when s/he had to leave his/her attachment figures or when s/he knew it was about to happen?	0	1	2
k)	has being apart from his/her attachment figures, or the thought of being apart from them led to worry, crying, tantrums, clinginess or misery?	0	1	2

If any of the items in A3 have been answered "A lot more than others" then tick Separation Anxiety on the check list in M1 (p.55) and continue with A4. If not, skip to section B.

A4 Have his/her worries about separation been there for at least **4 weeks**?

No	Yes
0	1

A5 How old was s/he when his/her worries about separation began?

(if since birth, enter 0)

[ ] years old

A6 How much have these worries upset or distressed him/her?

Not at all	A little	A medium amount	A great deal
0	1	2	3

A7 Have these worries interfered with ...

		Not at all	A little	A medium amount	A great deal
a)	how well s/he gets on with you and the rest of the family?	0	1	2	3
b)	making and keeping friends?	0	1	2	3
c)	learning or class work?	0	1	2	3
d)	playing, hobbies, sports or other leisure activities?	0	1	2	3

A8 Have these worries put a burden on you or the family as a whole?

Not at all	A little	A medium amount	A great deal
0	1	2	3

Section B Fears of specific things or situations

This section of the interview is about some things or situations that children are often scared of, even though they aren't really a danger to them. I'd like to know what [Name] is afraid of. I am interested in how s/he is usually - not on the occasional 'off day'. Not all fears are covered in this section – some are covered in other sections, e.g. fears of social situations, dirt, separation, crowds.

B1	Is [Name] scared of any of the things or situations on this list?	No	A little	A lot
a)	Animals: Dogs, spiders, bees and wasps, mice and rats, snakes, or any other animal, bird or insect	0	1	2
b)	Some aspect of the natural environment, e.g. storms, thunder, heights, water	0	1	2
c)	The dark	0	1	2
d)	Loud noises, e.g. fire alarms, fireworks	0	1	2
e)	Blood - injection - injury: Set off by the sight of blood or injury, or by an injection, or by other medical procedures	0	1	2
f)	Dentists or doctors	0	1	2
g)	Vomiting, choking or getting particular diseases, e.g. cancer or AIDS	0	1	2
h)	Using particular types of transport, e.g. cars, buses, boats, planes, ordinary trains, underground trains, bridges	0	1	2
i)	Small enclosed spaces, e.g. lifts, tunnels	0	1	2
j)	Using the toilet, e.g. at school or in someone else's house	0	1	2
k)	Specific types of people, e.g. clowns, people with beards, with crash-helmets, in fancy dress, dressed as Santa Claus	0	1	2
l)	Imaginary or supernatural beings, e.g. monsters, ghosts, aliens, witches	0	1	2
m)	Any other specific fear *(Describe)*	0	1	2

If any of the items in B1 have been answered "a lot", then continue with B2. Otherwise, go to section C.

B2	Are these fears a real nuisance to him/her, to you, or to anyone else?	No	Perhaps	Definitely
		0	1	2

If B2 = "Definitely" or if SDQ emotion score is ≥ 4 then continue. If neither, then skip to section C.

B3 How long has this fear or the most severe of these fears been present?

Less than 1 month	1 - 5 months	6 months or more
0	1	2

B4 When [Name] comes up against the things s/he is afraid of, or when s/he thinks s/he is about to come up against them, does s/he become anxious or upset?

No	A little	A lot
0	1	2
	B7	↓ B5

B5 Does s/he become anxious or upset every time, or almost every time, s/he comes up against the things s/he is afraid of?

No	Yes
0	1

B6 How often do his/her fears result in his/her becoming upset like this?

N.B. if [Name] is afraid of something that is only there for part of the year (e.g. wasps), this question is about that particular season.

Every now and then	Most weeks	Most days	Many times a day
0	1	2	3

B7 Do his/her fears lead to him/her avoiding the things s/he is afraid of?

No	A little	A lot
0	1	2
	B9	↓ B8

B8 Does this avoidance interfere with his/her daily life?

No	A little	A lot
0	1	2

	No	Perhaps	Definitely
B9 Do <u>you</u> think that his/her fears are over the top or unreasonable?	0	1	2
B10 And what about him/her? Does <u>s/he</u> think that his/her fears are over the top or unreasonable?	0	1	2

If B2 = "Definitely" or B4 = "A lot" or B7 = "A lot", then tick Specific Phobia on check list in M1(p.55).

B11 Have his/her fears put a burden on you or the family as a whole?

Not at all	A little	A medium amount	A great deal
0	1	2	3

Section C Fear of social situations

I am interested in whether (Child) is particularly afraid of social situations. This is as compared with other children of his/her age, and is not counting the occasional 'off day' or ordinary shyness.

C1	Overall, does [Name] particularly fear or avoid social situations that involve a lot of people, meeting new people, or doing things in front of people?	No	Yes
		0	1

If C1 = "Yes" or if SDQ emotion score is ≥ 4, then continue. If neither, then skip to section D.

C2	Has [Name] been particularly afraid of any of the following social situations over the **last 4 weeks**?	No	A little	A lot
a)	Meeting new people?	0	1	2
b)	Meeting a lot of people, such as at a party?	0	1	2
c)	Eating in front of others?	0	1	2
d)	Speaking in class?	0	1	2
e)	Reading out loud in front of others?	0	1	2
f)	Writing in front of others?	0	1	2

If none of the items in C2 have been answered "A lot", then skip to section D.

C3 Most children are attached to a few key adults, feeling more secure when they are around. Some children are only afraid of social situations if they don't have one of these key adults around.

Other children are afraid of social situations even when they are with one of these key adults.

Which is true for him/her?	Mostly fine in social situations as long as key adults are around	Social fears are marked even when key adults are around
	0	1

C4 Is [Name] just afraid with adults, or is s/he also afraid in situations that involve a lot of children, or meeting new children?

Just with adults	Just with children	With both adults and children
0	1	2

C5 Outside of these social situations, is [Name] able to get on well enough with the adults and children s/he knows best?

No	Yes
0	1

C6 Do you think his/her dislike of social situations is because s/he is afraid s/he will act in a way that will be embarrassing or show him/her up?

No	Perhaps	Definitely
0	1	2

C7 *(Only ask if C2d = 'A lot', or C2e = 'A lot', or C2f = 'A lot')*

Is his/her dislike of social situations related to specific problems with speech, reading or writing?

No	Perhaps	Definitely
0	1	2

C8 How long has his/her fear of social situations been present?

Less than 1 month	1 - 5 months	6 months or more
0	1	2

C9 How old was s/he when this fear of social situations began?

(if since birth, enter 0)

[ ] years old

C10 When [Name] is in one of the social situations s/he fears, or when s/he thinks s/he is about to come up against one of these situations, does s/he become anxious or upset?

No	A little	A lot
0	1	2

C12 ↓ C11

C11 How often does his/her fear of social situations result in him/her becoming upset like this?

Every now and then	most weeks	most days	many times a day
0	1	2	3

C12 Does his/her fear lead to him/her avoiding social situations?

No	A little	A lot
0	1	2

C14 ↓ C13

C13 Does this avoidance interfere with his/her daily life?

No	A little	A lot
0	1	2

	No	Perhaps	Definitely
C14 Does s/he think that this fear of social situations is over the top or unreasonable?	0	1	2
C15 Is s/he upset about having this fear?	0	1	2

If C10 = "A lot" or C12 = "A lot", then tick Social Phobia on check list in M1(p.55).

C16 Has his/her fear of social situations put a burden on you or the family as a whole?

Not at all	A little	A medium amount	A great deal
0	1	2	3

Section D Panic Attacks and Agoraphobia

Many children have times when they get very anxious or worked up about silly little things, but some children get severe panics that come out of the blue - they just don't seem to have any trigger at all.

D1	In the **last 4 weeks**, has [Name] had a panic attack when s/he suddenly became very panicky for no reason at all, without even a little thing to set him/her off?	No	Yes
		0	1

If D1 = "Yes" then tick the box for Panic on the check list in M1 (p.55).

D2	Over the **last 4 weeks**, has [Name] been very afraid of, or tried to avoid, the following situations?	No or Not Applicable	Yes
a)	Crowds	0	1
b)	Public Places	0	1
c)	Travelling alone (If s/he ever does so)	0	1
d)	Being far from home	0	1

If any of the items in D2 have been answered "Yes", then tick the box for Agoraphobia on the check list in M1 (p.55) and continue with D3. Otherwise skip to section E.

D3	Do you think this fear or avoidance of (Situation) is because s/he is afraid that if s/he had a panic attack, or something like that, s/he would find it difficult or embarrassing to get away, or wouldn't be able to get the help s/he needs?	No	Yes
		0	1

Section E Post Traumatic Stress

The next section is about events or situations that are exceptionally stressful, and that would really upset almost anyone. For example being caught in a burning house, being abused, being in a serious car crash or seeing you being mugged at gunpoint.

		No	Yes
E1	During his/her lifetime has anything like this happened to him/her?	0	1

If E1 = "Yes" then continue. Otherwise skip to section F.

		No	Yes
E2	Has [Name] ever experienced any of the following? (use card)		
	<u>Child involved in a disaster</u>		
a)	A serious and frightening accident, e.g. being run over by a car, being in a bad car or train crash, etc.	0	1
b)	A bad fire, e.g. trapped in a burning building	0	1
c)	Other disasters, e.g. kidnapping, earthquake, war	0	1
	<u>Violence to child</u>		
d)	A severe attack or threat, e.g. by a mugger or a gang	0	1
e)	Severe physical abuse that s/he still remembers	0	1
	<u>Sexual assault of child</u>		
f)	Sexual abuse	0	1
g)	Rape	0	1
	<u>Child witnessed something very upsetting</u>		
h)	Witnessed severe domestic violence, e.g. saw mother being badly beaten up at home	0	1
i)	Saw a family member or a friend severely attacked or threatened, e.g. by a mugger or a gang	0	1
j)	Witnessed a sudden death, a suicide, an overdose, a serious accident, a heart attack etc.	0	1
	<u>Other severe trauma</u>		
k)	Some other severe trauma (Describe) …… ……	0	1

If any of the items in E2 have been answered "Yes", then continue with E3. Otherwise, go to section F.

		No	Yes
E3	At the time, was [Name] very distressed or did his/her behaviour change dramatically?	0	1

		No	Yes
E3A	At present, is it affecting his/her behaviour, feelings or concentration?	0	1
		↓ Section F	↓ E4

		No	A little	A lot
E4	Over the **last 4 weeks**, has [Name]...			
a)	"relived" the event with vivid memories (flashbacks) of it?	0	1	2
b)	had repeated distressing dreams of the event?	0	1	2
c)	got upset if anything happened that reminded him/her of it?	0	1	2
d)	tried to avoid thinking or talking about anything to do with the event?	0	1	2
e)	tried to avoid activities, places or people that remind him/her of the event?	0	1	2
f)	blocked out important details of the event from his/her memory?	0	1	2
g)	shown much less interest in activities s/he used to enjoy?	0	1	2
h)	felt cut off or distant from others?	0	1	2
i)	expressed a smaller range of feelings than in the past, e.g. no longer able to express loving feelings?	0	1	2
j)	felt less confidence in the future?	0	1	2
k)	had problems sleeping?	0	1	2
l)	felt irritable or angry?	0	1	2
m)	had difficulty concentrating?	0	1	2
n)	always been on the alert for possible dangers?	0	1	2
o)	jumped at little noises or been easily startled in other ways?	0	1	2

If any part of E4 is answered "A lot", then tick the box for Post Traumatic Stress on the check list in M1 (p.55) and continue with E5. Otherwise, skip to section F.

E5 You have told me about (Definite Symptom/s). How long after the stressful event(s) did these other problems begin?

Within 6 months	More than 6 months after event
0	1

E6 How long has s/he been having these problems?

Less than 1 month	1 or 2 months	3 months or more
0	1	2

E7 How upset or distressed is s/he by the problems that the stressful event(s) triggered off?

Not at all	A little	A medium amount	A great deal
0	1	2	3

E8 Have these problems interfered with ...

		Not at all	A little	A medium amount	A great deal
a)	how well s/he gets on with you and the rest of the family?	0	1	2	3
b)	making and keeping friends?	0	1	2	3
c)	learning or class work?	0	1	2	3
d)	playing, hobbies, sports or other leisure activities?	0	1	2	3

E9 Have these problems put a burden on you or the family as a whole?

Not at all	A little	A medium amount	A great deal
0	1	2	3

Section F Compulsions and Obsessions

Many children have some rituals or superstitions, e.g. not stepping on the cracks in the pavement, having to go through a special goodnight ritual, having to wear lucky clothes for exams, or needing a lucky mascot for school sports matches. It is also common for children to go through phases when they seem obsessed by one particular subject or activity, e.g. cars, a pop group, a football team. But what I want to know is whether [Name] has any rituals or obsessions that go beyond this.

F1 Does [Name] have rituals or obsessions that upset him/her, waste a lot of his/her time, or interfere with his/her ability to get on with everyday life?

No	Yes
0	1

If F1 = Yes, or SDQ Emotion score is ≥ 4 then continue. If neither, then skip to section G.

F2 Over the **last 4 weeks**, has s/he had any of the following rituals (doing any of the following things over and over again even, though s/he has already done them or doesn't need to do them at all)?

		No	A little	A lot
a)	Excessive cleaning: hand washing, baths, showers, toothbrushing etc.	0	1	2
b)	Other special measures to avoid dirt, germs or poisons	0	1	2
c)	Excessive checking: electric switches, gas taps, locks, doors, the oven	0	1	2
d)	Repeating the same simple activity many times in a row for no reason, e.g. repeatedly standing up or sitting down or going backwards and forwards through a doorway	0	1	2
e)	Touching things or people in particular ways	0	1	2
f)	Arranging things so they are just so, or exactly symmetrical	0	1	2
g)	Counting to particular lucky numbers or avoiding unlucky numbers	0	1	2

F3 Over the **last 4 weeks**, has [Name] been obsessively worrying about dirt, germs or poisons – not being able to get thoughts about them out of his/her mind?

No	A little	A lot
0	1	2

If any of the items in F2 or F3 have been answered "A lot", then tick Obsessions and Compulsions on the check list in M1 (p.55).

F4 Over the **last 4 weeks**, has [Name] been obsessed by the worry that something terrible will happen to him/her or to others, e.g. illnesses, accidents, fires?

No	A little	A lot
0	1	2

Skip rule at the start of F7 ↓ F6

F6 Is this obsession about something terrible happening to him/her or to others just one part of a general concern about being separated from his/her key attachment figures, or is it a problem in its own right?

Part of separation anxiety	A problem in its own right
0	1

If F6 = "A problem in its own right" then tick Obsessions and Compulsions on the check list in M1 (p.55).

F7 *If the Obsessions and Compulsions box is ticked in M1, then continue.*
Otherwise skip to section G

Have his/her rituals or obsessions been present on most days for a period of at **least 2 weeks**?

No	Yes
0	1

F8 Does s/he think that his/her rituals or obsessions are over the top or unreasonable?

No	Perhaps	Definitely
0	1	2

F9 Does s/he resist the rituals or obsessions?

No	Perhaps	Definitely
0	1	2

F10 Do the rituals or obsessions upset him/her?

No, s/he enjoys them	Neutral, s/he neither enjoys them nor becomes upset	They upset him/her a little	They upset him/her a lot
0	1	2	3

F11 Do the rituals or obsessions use up at least an hour a day on average?

No	Yes
0	1

F12 Have the rituals or obsessions interfered with…

		Not at all	A little	A medium amount	A great deal
a)	how well s/he gets on with you and the rest of the family?	0	1	2	3
b)	making and keeping friends?	0	1	2	3
c)	learning or class work?	0	1	2	3
d)	playing, hobbies, sports or other leisure activities?	0	1	2	3

F13 Have the rituals or obsessions put a burden on you or the family as a whole?

Not at all	A little	A medium amount	A great deal
0	1	2	3

Section G Generalized Anxiety

This section is about worrying

G2 Does [Name] ever worry?

No	Yes
0	1
↓	↓
Section H	Continue

G2A Some children worry about just a few things, sometimes related to specific fears, obsessions or separation anxieties. Other children worry about many different aspects of their lives. They may have specific fears, obsessions or separation anxieties, but they also have a wide range of worries about many things.

Is [Name] a worrier in general?

No, s/he just has a few specific worries	Yes, s/he worries in general
0	1
↓	↓
Only continue if SDQ emotion score ≥ 4	*Continue*

G3 Over the **last 6 months**, has [Name] worried so much about so many things that it has really upset him/her or interfered with his/her life?

No	Perhaps	Definitely
0	1	2

If G3 = "Perhaps" or G3 = "Definitely" or SDQ emotion score is ≥ 4, then continue. If neither, then skip to section H.

G4	Over the **last 6 months**, and by comparison with other children of the same age, has [Name] worried about…	No more than others	A little more than others	A lot more than others
a)	Past behaviour: Did I do that wrong? Have I upset someone? Have they forgiven me?	0	1	2
b)	School work, homework or examinations	0	1	2
c)	Disasters: Burglaries, muggings, fires, bombs etc.	0	1	2
d)	His/her own health	0	1	2
e)	Bad things happening to others: family, friends, pets, the world (e.g. wars).	0	1	2
f)	The future: e.g. changing school, moving house, getting a job, getting a boy/girlfriend	0	1	2
g)	Making and keeping friends	0	1	2
h)	Death and dying	0	1	2
i)	Being bullied or teased	0	1	2
j)	His/Her appearance or weight	0	1	2
k)	Other specific worry (Describe) …… ……	0	1	2

If 2 or more of these worries were answered 'a lot more than others' then continue, else skip to section H

G6	Over the **last 6 months** has s/he worried excessively on more days than not?	No	Yes
		0	1

G7	Does s/he find it difficult to control the worry?	No	Yes
		0	1

If G6 = "Yes" or G7 = "Yes" then tick Generalized Anxiety on the check list in M1 (p.55) and continue. If neither are "Yes" then skip to section H.

G8 *If any of the following questions are answered "Yes", ask* "Has this been true for more days than not in the **last 6 months**?" *and record answer in second column.*

		In general			***More days than not in last 6 months***	
		No	Yes		No	Yes
a)	Does worrying lead to him/her feeling restless, keyed up, on edge, or unable to relax?	0	1	→	0	1
b)	Does worrying lead to him/her feeling tired or worn out more easily?	0	1	→	0	1
c)	Does worrying lead to difficulties in concentrating or his/her mind going blank?	0	1	→	0	1
d)	Does worrying lead to irritability?	0	1	→	0	1
e)	Does worrying lead to muscle tension?	0	1	→	0	1
f)	Does worrying interfere with his/her sleep, e.g. difficulty in falling or staying asleep, or restless, unsatisfying sleep?	0	1	→	0	1

G9 How upset or distressed is [Name] as a result of all his/her various worries?

Not at all	A little	A medium amount	A great deal
0	1	2	3

G10 Have his/her worries interfered with...

		Not at all	A little	A medium amount	A great deal
a)	how well s/he gets on with you and the rest of the family?	0	1	2	3
b)	making and keeping friends?	0	1	2	3
c)	learning or class work?	0	1	2	3
d)	playing, hobbies, sports or other leisure activities?	0	1	2	3

G11 Have these worries put a burden on you or the family as a whole?

Not at all	A little	A medium amount	A great deal
0	1	2	3

Section H Depression

This section of the interview is about his/her mood.

H1 In the **last 4 weeks**, have there been times when [Name] has been very sad, miserable, unhappy or tearful?

No	Yes
0	1
↓	↓
H7	H2

H2 Over the **last 4 weeks**, has there been a period when s/he has been really miserable nearly <u>every</u> day?

No	Yes
0	1

H3 During the time when s/he has been miserable, has s/he been really miserable for <u>most</u> of the day?
(i.e. for more hours than not)

No	Yes
0	1

H4 When s/he has been miserable, could s/he be cheered up?

Easily	With difficulty/ only briefly	Not at all
0	1	2

H5 Over the **last 4 weeks**, the period of being really miserable has lasted:

Less than 2 weeks	2 weeks or more
0	1

If H1 = "Yes" <u>and</u> H2 = "Yes" <u>and</u> H3 = "Yes", then tick Depression on check list in M1 (p.55).

H7 In the **last 4 weeks**, have there been times when [Name] has been grumpy or irritable in a way that has been out of character for him/her?

No	Yes
0	1
↓	↓
H13	H8

H8 Over the **last 4 weeks**, has there been a period when s/he has been really grumpy or irritable nearly <u>every</u> day?

No	Yes
0	1

H9 During the period when s/he has been grumpy or irritable, has s/he been like that for <u>most</u> of the day? (i.e. for more hours than not)

No	Yes
0	1

H10 Has the irritability been improved by particular activities, by friends coming round, or by anything else?

Easily	With difficulty/ only briefly	Not at all
0	1	2

H11 Over the **last 4 weeks**, the period of being really irritable has lasted:

Less than 2 weeks	2 weeks or more
0	1

If H7 = "Yes" <u>and</u> H8 = "Yes" <u>and</u> H9 = "Yes", then tick Irritability on check list in M1 (p.55).

H13 In the **last 4 weeks**, have there been times when [Name] has lost interest in everything, or nearly everything, that s/he normally enjoys doing?

No	Yes
0	1
↓ *Skip rule at start of H18*	↓ H14

H14 Over the **last 4 weeks**, has there been a period when this lack of interest has been present nearly <u>every</u> day?

No	Yes
0	1

H15 During these days when s/he has lost interest in things, has s/he been like this for <u>most</u> of each day?
(i.e. for more hours than not)

No	Yes
0	1

H16 Over the **last 4 weeks**, this loss of interest has lasted:

Less than 2 weeks	2 weeks or more
0	1

H17 *If Depression or Irritability box has been checked, ask:*

Has this loss of interest been present during the same period when s/he has been really miserable or irritable for most of the time?

No	Yes
0	1

If H13 = "Yes" <u>and</u> H14 = "Yes", then tick Loss of Interest on check list in M1 (p.55).

H18 *If Depression or Irritability or Loss of Interest box has been ticked on the check list M1 (p.55), then continue. Otherwise skip to H22.*

	During the period when [Name] was sad, irritable or lacking in interest…	No	Yes
a)	did s/he lack energy or seem tired all the time?	0	1
b)	was s/he eating much more or much less than normal?	0	1
c)	did s/he either lose or gain a lot of weight?	0	1
d)	did s/he find it hard to get to sleep or to stay asleep?	0	1
e)	did s/he sleep too much?	0	1
f)	was s/he agitated or restless for much of the time?	0	1
g)	did s/he feel worthless or unnecessarily guilty for much of the time?	0	1
h)	did s/he find it unusually hard to concentrate or to think things out?	0	1
i)	did s/he think about death a lot?	0	1
j)	did s/he talk about harming himself/herself or killing himself/herself?	0	1
k)	did s/he try to harm himself/herself or kill himself/herself?	0	1

H18L Over the whole of his/her lifetime, has s/he ever tried to harm himself/herself or kill himself/herself?

No	Yes
0	1

H19 How much has his/her sadness, irritability or loss of interest upset or distressed him/her?

Not at all	A little	A medium amount	A great deal
0	1	2	3

		Not at all	A little	A medium amount	A great deal
H20	Has his/her sadness, irritability or loss of interest interfered with...				
a)	how well s/he gets on with you and the rest of the family?	0	1	2	3
b)	making and keeping friends?	0	1	2	3
c)	learning or class work?	0	1	2	3
d)	playing, hobbies, sports or other leisure activities?	0	1	2	3

		Not at all	A little	A medium amount	A great deal
H21	Has his/her sadness, irritability or loss of interest put a burden on you or the family as a whole?	0	1	2	3

Now go to section J. ***Do not ask*** *H22 to H24 if you have already asked H18 i to l.*

Deliberate Self-Harm

		No	Yes
H22	Over the **last 4 weeks**, has s/he talked about deliberately harming or hurting himself/herself?	0	1
H23	Over the **last 4 weeks**, has s/he tried to harm or hurt himself/herself?	0	1
H24	Over the whole of his/her lifetime, has s/he ever tried to harm or hurt himself/herself?	0	1

If H22="Yes" or H23="Yes" or H24="Yes", then tick Deliberate Self-Harm on check list in M1 (p.55).

Section J Attention and Activity

This section of the interview is about his/her level of activity and concentration over the **last 6 months**. Nearly all children are overactive or lose concentration at times, but what I would like to know is how [Name] compares with other children of his/her own age. I am interested in how s/he is usually - not on the occasional 'off day'.

J1 Allowing for his/her age, do you think that [Name] definitely has some problems with overactivity or poor concentration?

No	Yes
0	1

If J1 = "Yes" or if SDQ hyperactivity score is ≥6, then continue. If neither, then skip to section K.

J2 I would now like to go through some more detailed questions about how [Name] has usually been over the **last 6 months**. I will start with questions about how active s/he has been.

	Over the **last 6 months**, and compared with other children of his/her age...	No more than others	A little more than others	A lot more than others
a)	Does s/he often fidget?	0	1	2
b)	Is it hard for him/her to stay sitting down for long?	0	1	2
c)	Does s/he run or climb about when s/he shouldn't?	0	1	2
d)	Does s/he find it hard to play or take part in other leisure activities without making a lot of noise?	0	1	2
e)	If s/he is rushing about, does s/he find it hard to calm down when someone asks him/her to?	0	1	2

J3 The next few questions are about impulsiveness.

	Over the **last 6 months**, and compared with other children of his/her own age…	No more than others	A little more than others	A lot more than others
a)	Does s/he often blurt out an answer before s/he had heard the question properly?	0	1	2
b)	Is it hard for him/her to wait his/her turn?	0	1	2
c)	Does s/he often butt in on other people's conversations or games?	0	1	2
d)	Does s/he often go on talking even if s/he has been asked to stop, or if no one is listening?	0	1	2

J4 The next set of questions are about attention.

	Over the **last 6 months**, and compared with other children his/her age…	No more than others	A little more than others	A lot more than others
a)	Does s/he often make careless mistakes or fail to pay attention to what s/he is supposed to be doing?	0	1	2
b)	Does s/he often seem to lose interest in what s/he is doing?	0	1	2
c)	Does s/he often not listen to what people are saying to him/her?	0	1	2
d)	Does s/he often not finish a job properly?	0	1	2
e)	Is it often hard for him/her to get himself/ herself organized to do something?	0	1	2
f)	Does s/he often try to get out of things s/he would have to think about, such as homework?	0	1	2
g)	Does s/he often lose things s/he needs for school or games?	0	1	2
h)	Is s/he easily distracted?	0	1	2
i)	Is s/he often forgetful?	0	1	2

	Have his/her teachers complained over the **last 6 months** of problems with...	No	A little	A lot
a)	fidgetiness, restlessness or overactivity?	0	1	2
b)	poor concentration or being easily distracted?	0	1	2
c)	acting without thinking about what s/he is doing, frequently butting in, or not waiting his/her turn?	0	1	2

If two or more of the items in J2, J3, or J4 have been answered "A lot more than others," then tick the box for Hyperactivity on the check list in M1 (p.55) and continue to J6. If not, skip to section K.

J6 Have his/her difficulties with activity or concentration been there for at least **6 months**?

No	Yes
0	1

J7 How old was s/he when his/her difficulties with activity or concentration began?
(if since birth, enter 0)

[ ] years old

J8 How much have his/her difficulties with activity or concentration upset or distressed him/her?

Not at all	A little	A medium amount	A great deal
0	1	2	3

J9 Have his/her difficulties with activity or concentration interfered with...

		Not at all	A little	A medium amount	A great deal
a)	how well s/he gets on with you and the rest of the family?	0	1	2	3
b)	making and keeping friends?	0	1	2	3
c)	learning or class work?	0	1	2	3
d)	playing, hobbies, sports or other leisure activities?	0	1	2	3

J10 Have these difficulties with activity or concentration put a burden on you or the family as a whole?

Not at all	A little	A medium amount	A great deal
0	1	2	3

Section K Awkward and Troublesome Behaviour

This next section of the interview is about behaviour. Nearly all children are awkward and difficult at times – not doing what they are told, being irritable or annoying, having temper outbursts, and so on. What I would like to know is how [Name] compares with other children of the same age. I am interested in how s/he is usually, and not just on occasional 'off days'.

K1 Thinking about the **last 6 months**, how does his/her behaviour compare with other children of his/her age?

Less awkward or troublesome than average	About average	More awkward or troublesome than average
0	1	2

If K1 = "More awkward or troublesome than average," or if SDQ conduct problems score is ≥ 3, then continue. If neither, then skip to section P.

Some children are awkward or annoying with just one person - perhaps with yourself or just one brother or sister. Other children are troublesome with a range of adults or children. The following questions are about how [Name] is in general, and not just with one person.

	Over the **last 6 months**, and as compared with other children of the same age, has s/he often…	No more than others	A little more than others	A lot more than others
a)	had temper outbursts?	0	1	2
b)	argued with grown-ups?	0	1	2
c)	taken no notice of rules, or refused to do as s/he is told?	0	1	2
d)	seemed to do things to annoy other people on purpose?	0	1	2
e)	blamed others for his/her own mistakes or bad behaviour?	0	1	2
f)	been touchy or easily annoyed?	0	1	2
g)	been angry and resentful?	0	1	2
h)	been spiteful?	0	1	2
i)	tried to get his/her own back on people?	0	1	2

If any of the items in K2 have been answered "A lot more than others", then tick Awkward Behaviour on the check list M1 (p.55) and continue with K3. If not, skip to K8.

K3 Have his/her teachers complained over the **last 6 months** of problems with this kind of awkward behaviour or disruptiveness in class?

No	A little	A lot
0	1	2

K4 Has his/her awkward behaviour been there for at least **6 months**?

No	Yes
0	1

K5 How old was s/he when this sort of awkward behaviour began?

(if since birth, enter 0)

[ ] years old

K6 Has his/her awkward behaviour interfered with…

		Not at all	A little	A medium amount	A great deal
a)	how well s/he gets on with you and the rest of the family?	0	1	2	3
b)	making and keeping friends?	0	1	2	3
c)	learning or class work?	0	1	2	3
d)	playing, hobbies, sports or other leisure activities?	0	1	2	3

K7 Has his/her awkward behaviour put a burden on you or the family as a whole?

Not at all	A little	A medium amount	A great deal
0	1	2	3

Continue with K8.

Behaviour that sometimes gets children into trouble.

K8 I'm now going to ask about behaviour that sometimes gets children into trouble, including dangerous, aggressive or antisocial behaviour. Please answer according to how s/he has been over the last year - I'm switching to the **last 12 months** for this next set of questions.

If any of the following questions are answered "Definitely*" ask* "Has this been going on for the **last 6 months**?" *and record answer in second column.*

As far as you know, over the **last 12 months**...	***Over the last 12 months***				***Last 6 months***	
	No	Perhaps	Definitely		No	Yes
a) has s/he often told lies in order to get things or favours from others, or to get out of having to do things s/he is supposed to do?	0	1	2	→	0	1
b) has s/he often started fights? (*Other than with brothers and sisters*)	0	1	2	→	0	1
c) has s/he often bullied or threatened people?	0	1	2	→	0	1
d) has s/he often stayed out after dark much later than s/he was supposed to?	0	1	2	→	0	1
e) has s/he stolen from the house, or from other people's houses, or from shops or school? *(This doesn't include very minor thefts, e.g. stealing his/her brother's pencil or food from the fridge)*	0	1	2	→	0	1
f) has s/he run away from home more than once, or ever stayed away all night without your permission?	0	1	2	→	0	1
g) has s/he often played truant (bunked off) from school?	0	1	2	→	0	1

If any of the items in K8 have been answered "Definitely", then tick Troublesome Behaviour on the check list in M1 (p.55).

K9 *If 13 or older and definitely playing truant in the past year, ask this question. Otherwise go to the skip rule at the beginning of K10)*

Did s/he start playing truant (bunking off) from school before s/he was 13?

No	Yes
0	1

K10 *Only continue if check list M1 (p.55) has been ticked for Awkward Behaviour or Troublesome Behaviour. Otherwise skip to section P.*

May I now ask you about a list of less common but potentially more serious behaviours. I have to ask all people all questions even when they are not likely to apply.

If any of the following questions are answered "Yes" then ask "Has this happened in the **last 6 months**?" *and record answer in second column.*

	As far as you know, have any of the following happened even once in the **last 12 months**?	***Over the last 12 months***			***Last 6 months***	
		No	Yes		No	Yes
a)	Has s/he used a weapon or anything that could seriously hurt someone? *(e.g. a bat, brick, broken bottle, knife, gun)*	0	1	→	0	1
b)	Has s/he really hurt someone or been physically cruel to them? *(e.g. has tied up, cut or burned someone).*	0	1	→	0	1
c)	Has s/he been really cruel on purpose to animals and birds?	0	1	→	0	1
d)	Has s/he deliberately started a fire? *(This is only if s/he intended to cause severe damage. This question is not about lighting campfires, or burning individual matches or pieces of paper)*	0	1	→	0	1
e)	Has s/he deliberately destroyed someone else's property? *(This question is not about fire setting or very minor acts, e.g. destroying sister's drawing. It does include behaviour such as smashing car windows or school vandalism)*	0	1	→	0	1
f)	Has s/he been involved in stealing on the streets, e.g. snatching a handbag or mugging?	0	1	→	0	1
g)	Has s/he tried to force someone to have sexual activity against their will?	0	1	→	0	1
h)	Has s/he broken into a house, any other building or a car?	0	1	→	0	1

If any of the items in K10 have been answered "Yes", then tick Troublesome Behaviour on the check list in M1 (p.55).

K11 Have his/her teachers complained of troublesome behaviour over the **last 6 months**?

No	Yes
0	1

K11AA Has his/her troublesome behaviour been present for at **least 6 months?**

No	Yes
0	1

K11A Has [Name] ever been in trouble with the police? *(Describe)*

.....

.....

No	Yes
0	1

If K11A= "Yes" then tick Trouble With The Police on the check list in M1 (p.55).

If the check list in M1 (p.55) has been ticked for Troublesome Behaviour then continue. Otherwise skip to section P.

K12	Has his/her troublesome behaviour interfered with…	Not at all	A little	A medium amount	A great deal
a)	how well s/he gets on with you and the rest of the family?	0	1	2	3
b)	making and keeping friends?	0	1	2	3
c)	learning or class work?	0	1	2	3
d)	playing, hobbies, sports or other leisure activities?	0	1	2	3

K13	Has his/her troublesome behaviour put a burden on you or the family as a whole?	Not at all	A little	A medium amount	A great deal
		0	1	2	3

Section P Dieting, weight and body shape

P1		No	Yes
a)	Has [Name] ever thought s/he was fat even when other people said s/he was *very* thin?	0	1
b)	Would [Name] be ashamed if other people knew how much s/he eats?	0	1
c)	Has [Name] ever deliberately made him/herself vomit (throw up)?	0	1
d)	Do worries about eating (what? where? how much?) really interfere with his/her life?	0	1
e)	If [Name] eats too much, does s/he blame him/herself a lot?	0	1

If two or more of the P1 questions is answered 'Yes' continue. Otherwise skip to next section

P2a	How tall is [Name]? (approximately)	☐	cms
		☐☐	feet + inches
P2b	How much does [Name] weigh at present? (approximately)	☐	kg
		☐	pounds
		☐☐	stones + pounds
P2c	What was his/her lowest weight in the last 12 months?	☐	kg
		☐	pounds
		☐☐	stones + pounds
P2d	What was his/her highest weight ever? (*excluding pregnancy)*	☐	kg
		☐	pounds
		☐☐	stones + pounds

P3 At present, would you describe him/her as very thin, thin, average, plump or fat?

Very thin	Thin	Average	Plump	Fat
0	1	2	3	4
↓	↓	⌣		
P4		Skip to P5		

P4 Comparing how s/he is this year with how s/he has been in previous years, would you say s/he was even thinner in previous years, always this thin, a little thinner this year than in previous years, or a lot thinner this year than in previous years?

Even thinner in previous years	Always this thin	A little thinner this year than in previous years	A lot thinner this year than in previous years
0	1	2	3

P5 At present, would s/he describe him/herself as very thin, thin, average, plump or fat?

Very thin	Thin	Average	Plump	Fat
0	1	2	3	4

If P3 = 'Very thin' or P5 = 'Very thin', then tick 'Very Thin' on the check list in M1 (p.55).

P6 Have you or other people – family, a friend, a doctor – been seriously concerned that his/her weight has been bad for his/her physical health?

No	Yes
0	1

P7 What does [Name] think? Does s/he think that his/her weight has been bad for his/her physical health?

No	Yes
0	1

P8 Is [Name] afraid of gaining weight or getting fat?

No	A little	A lot
0	1	2
⌣		↓
P10		P9

P9 Does the thought of gaining weight or getting fat really terrify him/her?

No	Yes
0	1

P10 If a doctor told him/her that s/he needed to put on five pounds (two kilograms) for the sake of her health, would s/he find this easy, difficult or impossible to accept?
(If a child has physical problems that make it hard for him/her to put on weight, the question is whether s/he is willing to try, not whether s/he can succeed.)

Easy	Difficult	Impossible
0	1	2

P11 Does [Name] avoid the sorts of food that s/he things will make him/her fat?

No	A little	A lot
0	1	2
	P13	↓ P12

P12 How often does [Name] succeed in this? (i.e. avoiding fattening food)

Never	Sometimes	Most of the time	Always
0	1	2	3

P13 Does [Name] spend a lot of his/her time thinking about food?

No	Yes
0	1

P14 Sometimes people say that they have such a strong desire for food, and that this desire is so hard to resist, that it is like the way an addict feels about drugs or alcohol.

Does this apply to him/her?

No	A little	A lot
0	1	2

If P9 = 'Yes or P10 = 'Impossible' or P14 = 'A lot', then tick 'Focus on Weight and Food' on the check list in M1 (p.55).

P15 Sometimes people lose control over what they eat, and then they eat a very large amount of food in a short time. For example, they may open the fridge and eat as much as they can find – eating and eating until they feel physically ill. This usually happens when people are by themselves.

Does this happen to him/her?

No	Yes
0 ↓ P18	1 ↓ *Tick 'Loss of Control' on the check list in M1 (p.55) and continue with P16*

P16 Over the last three months, how often on average has this happened?

Hasn't happened	Occasionally	About once a week	Twice a week or more
0	1	2	3

P17 When this happens, does [Name] have a sense of having lost control over his/her eating?

No	Yes
0	1

P17a) Please describe how much s/he typically eats during one of his/her episodes of eating too much ('binge'):

P18 Over the last three months, has [Name] done any of the following to avoid putting on weight:

	(When "No" check if the child tries but is not allowed)	No	Tries to but not allowed	A little	A lot
a)	Eating less at meals	0	1	2	3
b)	Skipping meals	0	1	2	3
c)	Going without food for long periods, e.g. all day or most of the day	0	1	2	3
d)	Hiding or throwing away food that others give him/her	0	1	2	3
e)	Exercising more	0	1	2	3
f)	Making him/herself vomit (throw up)	0	1	2	3
g)	Taking pills or medicines in order to lose weight Please describe: …….	0	1	2	3
h)	Doing other things *(e.g. not taking insulin if diabetic).* Please describe: …… ……..…	0	1	2	3

If the answer to any of the parts of P18 is 'a lot', tick 'Avoidance of Weight Gain' on the check list in M1 (p.55). If the 'Loss of Control' checkbox is also ticked, then continue with P19. Otherwise skip to P20 for females or P26 for males.

P19 You told me earlier about the times when [Name] loses control and eats too much. After s/he does this, does s/he normally then (restrict eating/ exercise/ vomit/ take pills or medicine) to stop him/herself putting on weight?

No	Yes
0	1

Skip to P26 for males.

P20 Has she had any periods in the last three months?

No	Yes
0	1
↓	↓
P21	*P22*

P21 Has she ever had any periods?

No	Yes
0	1
↓	↓
P26	*P23*

P22 Is she taking any hormone pills or injections? (including contraceptives)

No	Yes
0	1
↓	↓
Continue with P23 in all cases	

P23) Please describe how her periods have been in general, and how they have been recently.

If P20 was 'No' and P21 was 'Yes', ask:
P24) Why do you think she has not had any periods in the last 3 months?

If P22 was 'Yes', ask:
P25) Please describe what effects the hormone pills or injections have on her periods.

P26 ***Skip rule before starting P26**: If 'Very Thin' or 'Focus on Weight and Food' or 'Loss of Control' or 'Avoidance of Weight Gain' has been ticked on the check list M1 (p.55), then continue. Otherwise skip to the next section.*

	Not at all	A little	A medium amount	A great deal
You have told me about his/her eating pattern and concern about weight or body shape. How upset or distressed is s/he by this?	0	1	2	3

P27	How much have his/her eating pattern or concern about weight and body shape interfered with...	Not at all	A little	A medium amount	A great deal
a)	how well s/he gets on with you and the rest of the family?	0	1	2	3
b)	making and keeping friends?	0	1	2	3
c)	learning or class work?	0	1	2	3
d)	playing, hobbies, sports or other leisure activities?	0	1	2	3

P28	Not at all	A little	A medium amount	A great deal
Has her eating pattern or concern about weight or body shape put a burden on you or the family as a whole?	0	1	2	3

Section Q Tics

Q1	Over the last 12 months, has [Name] had any tic **movements** that s/he couldn't seem to control – such as excessive eye blinking, facial grimaces, nose twitches or head nodding?	No	Yes
		0	1

Q2	Over the last 12 months, has [Name] had any tic **sounds** that s/he couldn't seem to control – such as excessive sniffing, coughing or throat clearing?	No	Yes
		0	1

If Q1 = "Yes" or Q2 = 'Yes' then continue. If both are 'No', then skip the rest of this section

Q3 What doctors mean by 'motor tics' are repeated movements that are sudden and rapid, that follow more or less the same pattern every time, and that occur without the person really wanting them to.

	Thinking about the whole of his/her life, has s/he ever had motor tics involving any of the following types of repeated movement?	No	Yes
a)	Excessive blinking of eyes	0	1
b)	Raising of eyebrows	0	1
c)	Screwing up eyes	0	1
d)	Rolling eyes up, down or sideways	0	1
e)	Twitching of nose	0	1
f)	Flaring of nostrils	0	1
g)	Pouting of mouth (as if giving a kiss)	0	1
h)	Stretching mouth wide open	0	1
i)	Nodding of head	0	1
j)	Screwing up of face	0	1
k)	Touching chin to shoulder	0	1
l)	Stretching neck	0	1
m)	Shrugging shoulder	0	1
n)	Jerking movement of arm or leg	0	1
o)	Other motor tics (*Describe*:) …….…….. …….. ……..	0	1

If any of the parts of Q3 are answered 'Yes' then tick Motor Tics on the check list in M1 (p.55) and continue with the following, otherwise skip to Q6

Q4 Sometimes, movements that look like tics turn out to have some other explanation. For example, some children squint because they need to wear glasses or change to stronger glasses. Similarly some children have nose and eye problems during the hay fever season.

Do you think that some or all of his/her movements could have been caused by other things?

No	Yes
0	1
↓	↓
Q6	*Q5*

Q5 Please describe what other things might have caused his/her movements

……

……

……

Q6 We are now going to move on from motor tics to vocal tics. These are sounds that come from the mouth, nose or throat. They are sudden and rapid, they follow more or less the same pattern every time, and they occur without the person really wanting them to.

	Thinking about the whole of his/her life, has s/he ever had vocal tics involving any of the following types of repeated sounds?	No	Yes
a)	Throat clearing	0	1
b)	Excessive sniffing	0	1
c)	Coughing as a habit	0	1
d)	Gulping	0	1
e)	High-pitched squeaks	0	1
f)	Making little noises, e.g. ‘Ah’, ‘Eh’, ‘Eee’	0	1
g)	Sucking noises	0	1
h)	Burping, not just when eating or drinking	0	1
i)	A word said repeatedly and out of context	0	1
j)	Swearing, without meaning to and without being annoyed	0	1
k)	Other vocal tics (*Describe*:) ……..	0	1
	…….		

If any of the parts of Q6 are answered ‘Yes’ then tick Vocal Tics on the check list in M1 (p.55) and continue with the following, otherwise skip to Q9

Q7 Sometimes, sounds that seem like tics turn out to have some other explanation. For example, some children clear their throat when they are nervous or cough a lot because they have a tickly throat with a cold or hay fever.

Do you think that some or all of his/her sounds could have been caused by other things?

No	Yes
0	1
↓	↓
Q9	*Q8*

Q8 Please describe what other things might have caused his/her sounds

……

……

……

Skip rule before Q9: *Only continue if either Motor or Vocal Tics has been ticked in M1. Otherwise, skip the rest of this section.*

Q9 Do the tics go away when s/he is asleep?

No	Yes
0	1

Q10 Do the tics sometimes worsen when s/he relaxes, e.g. while watching TV after a busy day at school?

No	Yes
0	1

Q11 If [Name] tries really hard, can s/he stop the tics from happening?

No	Yes
0	1
↓	↓
Q13	*Q12*

Q12 If s/he uses her will power to keep the tics under control for a while, does s/he get a rebound later, e.g. fewer tics when visitors come, but an extra burst of them later when they've gone?

No	Yes
0	1

Q13 How old was s/he when the tics first began?

(if since birth, enter 0)

	years old

Q14 I am going to be asking next about bad weeks for tics. What I mean by a bad week for tics is one when the tics are happening many times a day, either every day that week or most days that week.

Over the last year, has [Name] had any bad weeks for tics?

(Optional:) Just to remind you, that means at least one week when s/he had many tics a day, either every day that week, or most days that week.

No	Yes
0	1
↓	↓
Go to next section	*Q15*

Q15 When did [Name] first start having bad weeks for tics?

Less than a month ago	1-11 months ago	At least a year ago
0	1	2
↓	⌣	⌣
Q21	Q16	

Q16 Over the last year, roughly how many weeks have been bad weeks for tics?

Well under half of them	About half of them	Well over half of them	All or nearly all of them
0	1	2	3

Q17 Over the last year, has [Name] had a period of at least 4 weeks in a row that were bad weeks for tics?

No	Yes
0	1
↓	↓
Q19	*Q18*

Q18 Have the last 4 weeks been bad weeks for tics?

No	Yes
0	1

Q19 Some children/young people have tics week in, week out – though the pattern and number of tics isn't necessarily the same every week.

Other children/young people have weeks or months when the tics go away completely

Over the last year, has [Name] had any tic-free periods lasting weeks or months?

No	Yes
0	1
↓	↓
Q21	*Q20*

Q20 What has been the longest tic-free period this year?

Up to 2 months	3 months	More than 3 months
0	1	2

Q21 How upset or distressed is [Name] as a result of all his/her tics?

Not at all	A little	A medium amount	A great deal
0	1	2	3

Q22 Have his/her tics interfered with...

		Not at all	A little	A medium amount	A great deal
a)	how well s/he gets on with you and the rest of the family?	0	1	2	3
b)	making and keeping friends?	0	1	2	3
c)	learning or class work?	0	1	2	3
d)	playing, hobbies, sports or other leisure activities?	0	1	2	3

Q23 Have the tics put a burden on you or the family as a whole?

Not at all	A little	A medium amount	A great deal
0	1	2	3

Section L Other concerns

*Do **NOT** ask if you are including the Development section (p.4 onwards) in this interview*

Ll	In his/her first 3 years of life, was there anything that seriously worried you about...	No	Yes
a)	the way his/her speech developed?	0	1
b)	how s/he got on with other people?	0	1
c)	any odd rituals or unusual habits that were very hard to interrupt?	0	1

*Do **NOT** ask unless L1a, L1b or L1c = Yes)*

L2	Have all these early delays or difficulties now cleared up completely?	Completely cleared up	Some continuing problems
		0	1

*Do **NOT** ask if you are including the Tics section (p.49 onwards) in this interview*

L3	Does s/he have any tics or twitches that s/he can't seem to control?	No	Yes
		0	1

*Do **NOT** ask if you are including the Eating Disorders section (p.43 onwards) in this interview*

L4	Have you been concerned about him/her being too thin or dieting too much?	No	Yes
		0	1

***ASK** in all cases*

L5	Apart from the things you have already told me about, are there any other aspects of his/her psychological development that really concern <u>you</u>?	No	Yes
		0	1

***ASK** in all cases*

L6	Apart from the things you have already told me about, are there any other aspects of his/her psychological development that really concern <u>his/her teachers</u>?	No	Yes
		0	1

If L2 = "Some continuing problems", or L3 = "Yes" or L4 = "Yes" or L5 = "Yes" or L6 = "Yes" then tick Other Concerns on the check list in M1 (p.55).

Section M Areas of Difficulty

M1 **Check list of difficulties**

Dev [ ] Development = difficulties with language, routines, play, or social ability

A [ ] Separation anxiety = fear of being separated from *(list from A1)*

B [ ] Specific phobia = fear of *(from B1)*

C [ ] Social phobia = fear of *(from C2)*

D [ ] Panic = panic attacks
[ ] Agoraphobia = avoidance of crowds, being out alone etc. *(from D2).*

E [ ] Post traumatic stress = distress triggered by his/her experience of *(from E2)*

F [ ] Obsessions and compulsions = rituals or obsessions involving *(from F2, F 3 and F4)*

G [ ] Generalized anxiety = excessive worrying about *(from G4)*

H [ ] Depression
[ ] Irritability
[ ] Loss of interest
[ ] Deliberate self-harm

J [ ] Hyperactivity = difficulties with activities and attention such as *(from J2, J3 and J4)*

K [ ] Awkward behaviour = awkward behaviours such as *(from K2)*
[ ] Troublesome behaviour = troublesome behaviour such as *(from K8 and K10)*
[ ] Trouble with the Police

P [ ] Very thin
[ ] Focus on weight and food
[ ] Loss of control
[ ] Avoidance of weight gain

Q [ ] Motor tics
[ ] Vocal tics

L [ ] Other concerns = Concerns about *(from L2, L3 L4 L5 and L6)*

M2 **Getting a description of the child's difficulties in the parent's own words**

If none of the boxes in M1 are ticked, skip to section N.

Whenever you have checked a box for one of the sections in M1, you should make sure that you get answers to the corresponding open-ended questions about that section. These open-ended questions are listed below as suggestions, but you can use your initiative to add extra questions or explain the existing questions more clearly.

You have a choice – you can ask the open-ended questions as you go along, or you can ask them after you have finished sections A to L. For example, if you tick the box for section A, then you could ask the extra questions before going on to section B, or you could wait until you have finished all the sections from A to L. If you are asking all the open-ended questions at the end, then it is often a good idea to let the parents choose which order to take the different topics in, starting with the area that concerns them most.

Whichever you decide to do, it is usually a good idea to note down the parents' spontaneous comments when they make them. That way, you will have less need to ask them to repeat themselves in this section. But do check before the end of the interview to make sure all questions have been covered for each area of difficulty.

When parents provide a vague or generalized answer, then ask them for specific examples. For example, if they say, "he worries about everything," then ask "What sorts of worries?" Or if they say, "he never does what he is told," then ask "Can you tell me about a recent occasion when he caused problems by not doing what he was told?"

Don't feel that you need to keep your answers short just because there's only a small space on this form – write small and use extra paper if necessary!

Introducing the open-ended questions:
You have already told me about his/her difficulties. I'd now like to hear a bit more about these in your own words.

M2R: Development

If M1Dev is ticked for development, ask:

M2R1 Please describe any aspects of his/her language, routines, play, or social ability that have concerned you at some point in his/her life.

M2R2 Are any of these difficulties interfering with his/her everyday life at present? If so, please describe what the current difficulties are, and how they are interfering with his/her life.

M2R3 Have these difficulties ever been given a diagnosis or label? If so, who suggested the diagnosis or label, and what was it?

M2R4 What help, if any, has s/he had for these difficulties?

M2A: Separation anxiety

If M1A is ticked for separation anxiety, ask

M2A1) Please describe his/her current worries about separation. How do these worries show themselves?

M2A2) How often does this worrying lead to difficulties?

M2A3) How severe are the difficulties at their worst?

M2A4) How long has he or she had these worries about separation?

M2A5) Are these worries interfering with his or her quality of life? If so, how?

M2A6) What do you think the worries are due to?

M2A7) Have you tried to do anything about these worries? If so, please describe what you've tried to do, any help that you have had, and whether this has made a difference.

M2B: Specific phobia

If M1B is ticked for specific phobia, ask

M2B1) Please describe any fears that are a real nuisance, that seriously upset him or her, or that lead to him or her not doing things that he or she would otherwise want to do.

M2B2) How often are his or her fears a nuisance or upsetting for him or her?

M2B3) How severe are the fears at their worst?

M2B4) Are his or her fears interfering with his or her quality of life? If so, how?

M2B5) Have you tried to do anything about these fears? If so, please describe what you've tried to do, any help that you have had, and whether this has made a difference.

M2C: Social phobia

If M2C is ticked for social phobia, ask

M2C1) Please describe any social fears that are a real nuisance, that seriously upset him or her, or that lead to him or her not doing things that he or she would otherwise want to do.

M2C2) How often do his or her social fears cause difficulties or upset him or her?

M2C3) How severe are these social fears at their worst?

M2C4) Are his or her social fears interfering with his or her quality of life? If so, how?

M2C5) Have you tried to do anything about these social fears? If so, please describe what you've tried to do, any help that you have had, and whether this has made a difference.

M2D: Panic/agoraphobia

If M1D is ticked for panic or agoraphobia, ask one or both of the following (according to whether the child has panic attacks or avoidance, or both)

M2D1) Please describe as fully as possible what these panic attacks are like, how often they occur, when they started, and what effect they have on his/her life.

M2D2) We'd like to hear more about his/her fear or avoidance of crowds, public places, travelling alone, or being far from home. Please describe this as fully as possible. Please also tell us how often this occurs, when it started, and what effect it has on his or her life.

M2E: Post traumatic stress

If M1E is ticked for post traumatic stress, ask

M2E1) What was the extremely stressful event? We're very sorry if asking about this is upsetting for you too. You only need to tell us enough details for us to make sense of his/her current symptoms.

M2E2) Please describe the symptoms that [Name] still has as a result of his or her very stressful experience.

M2E3) How often do these symptoms cause difficulties or upset him or her?

M2E4) How severe are the symptoms at their worst?

M2E5) Are the symptoms interfering with his or her quality of life? If so, how?

M2E6) Have you tried to do anything about these symptoms? If so, please describe what you've tried to do, any help that you have had, and whether this has made a difference.

M2F: Obsessions and compulsions

If M1F is ticked for obsessions and compulsions, ask

M2F1) Please describe all of his/her rituals or obsessions.

M2F2) How often do these rituals or obsessions cause difficulties or upset him or her?

M2F3) How severe are the rituals or obsessions at their worst?

M2F4) How long have they been present?

M2F5) Are they interfering with his or her quality of life? If so, how?

M2F6) Have you tried to do anything about these rituals or obsessions? If so, please describe what you've tried to do, any help that you have had, and whether this has made a difference.

M2G: Generalized anxiety

If M1G is ticked for generalized anxiety, ask

M2G1) Please describe what it is that [Name] worries about?

M2G2) How often does this worrying lead to difficulties?

M2G3) How severe are the worries at their worst?

M2G4) How long has he or she worried a lot about things?

M2G5) Are his or her worries interfering with his or her quality of life? If so, how?

M2G6) Have you tried to do anything about these worries? If so, please describe what you've tried to do, any help that you have had, and whether this has made a difference.

M2H: Depression

If M1H is ticked for depression, irritability or loss of interest, ask

M2H1) Please describe his/her mood (sadness, irritability) and his or her level of interest in things.

M2H2) What else has changed at the same time as his or her mood and level of interest? If relevant, tell us about energy, appetite, sleep, self-confidence, blaming him or herself, hopelessness about the future, thoughts of death, self-harm etc.

M2H3) Over the **last 4 weeks**, how much of the time has he or she been like this?

M2H4) Over the **last 4 weeks**, how severe have the difficulties been at their worst?

M2H5) When did this episode of low mood, irritability or loss of interest begin?

M2H6) What do you think triggered this episode off?

M2H7) Has he or she had similar episodes in the past? If so, please describe.

M2H8) Has he or she had episodes in the past when he or she has gone 'high' instead of 'low'? If so, please describe.

M2H9) Is his or her mood or loss of interest interfering with his or her quality of life? If so, how?

M2H10) Have you tried to do anything about his or her mood or loss of interest? If so, please describe what you've tried to do, any help that you have had, and whether this has made a difference.

M2H2: Deliberate self-harm

If M1H is ticked for deliberate self-harm, ask

M2H11) It would help us to hear more about his/her harming or hurting himself/herself , or at least talking about doing so.

M2J: Hyperactivity

If M1J is ticked for hyperactivity, ask

M2J1) Please describe difficulties that [Name] has with overactivity, lack of attention or impulsiveness.

M2J2) How often does his or her level of activity or his or her lack of attention lead to difficulties?

M2J3) How severe are the difficulties at their worst?

M2J4) How long has he or she been like this?

M2J5) Is his or her level of activity or his or her lack of attention interfering with his or her quality of life? If so, how?

M2J6) Have you tried to do anything about his or her overactivity, lack of attention or impulsiveness? If so, please describe what you've tried to do, any help that you have had, and whether this has made a difference.

M2K: Awkward and troublesome behaviour

If M1K is ticked for awkward or troublesome behaviour, ask

M2K1) Please describe his/her awkward and troublesome behaviour.

M2K2) How often does this behaviour lead to difficulties?

M2K3) How severe are the difficulties at their worst?

M2K4) How long has he or she been like this?

M2K5) Is his or her awkward and troublesome behaviour interfering with his or her quality of life? If so, how?

M2K6) Have you tried to do anything about his or her behaviour? If so, please describe what you've tried to do, any help that you have had, and whether this has made a difference.

M2P: Dieting, weight and body shape

If M1P is ticked for very thin, focus on weight and food, loss of control, or avoidance of weight gain, ask:

M2P1) Please describe any current concerns about his/her eating pattern, weight or body shape.

M2P2) Are the difficulties with food or weight due to a medical condition? If so, what is the condition?

M2P3) When did these concerns about food or weight start?

M2P4) Thinking about a typical day, please tell us what [Name] eats, what s/he avoids eating, and any calorie limit or rules that s/he uses to decide what to eat.

M2P5) Has his/her eating pattern or concern about his/her weight affected other aspects of his/her life? For example, reducing his/her interest in things that other people of his/her age enjoy, or affecting how well s/he gets on with family or friends.

M2P6) Has s/he, or have you or anyone else in the family, asked a doctor or a psychologist to help him/her with food or with his/her weight? If yes, what advice or help did you get? Did it help?

M2P7) Has [Name] had any medical problems related to his/her eating patterns, to his/her weight, or to the ways s/he controls his/her weight? (Include bleeding after vomiting, fainting, excessive weakness, constipation, visits to Accident and Emergency Departments, dental problems, etc.)

M2Q: Tics

If M1H is ticked for motor tics or vocal tics, ask:

M2Q1) Please describe his/her tics in your own words

M2Q2) How frequent and severe are they at their worst?

M2Q3) When and how did they start?

M2Q4) Are the tics interfering with his or her quality of life? If so, how?

M2Q5) Have you tried to do anything about the tics? If so, please describe what you've tried to do, any help that you have had, and whether this has made a difference.

M2L: Other concerns

If M1L is ticked for less common difficulties, ask whichever of the following apply:

M2L1) We would like to hear more about the sorts of difficulties that [Name] has with language, getting on with people, odd habits or unusual rituals.

M2L2) We would like to hear more about his or her tics or twitches.

M2L3) We would like to hear more about your concerns about his or her weight or dieting.

M2L4) We would like to hear more about the other things that you are concerned about.

M2L5) We would like to hear more about his or her teachers' concerns.

M2X: The interview in general:

M2X1) Finally, this is your opportunity as an interviewer to comment on the interview in general, e.g. to describe the level of motivation or understanding of the respondent, or to record your observations about the child's activity level while you were interviewing the child's parent.

Section N Strengths

I have been asking you a lot of questions about his/her difficulties and problems. I now want to ask you about his/her good points or strengths.

N1	Do the following descriptions apply to him/her?	No	A little	A lot
a)	Generous	0	1	2
b)	Lively	0	1	2
c)	Keen to learn	0	1	2
d)	Affectionate	0	1	2
e)	Reliable and responsible	0	1	2
f)	Easy going	0	1	2
g)	Good fun, good sense of humour	0	1	2
h)	Interested in many things	0	1	2
i)	Caring, kind-hearted	0	1	2
j)	Bounces back quickly after setbacks	0	1	2
k)	Grateful, appreciative of what s/he gets	0	1	2
l)	Independent	0	1	2

N2	What are the things s/he does that really please you?	No	A little	A lot
a)	Helps around the home	0	1	2
b)	Gets on well with the rest of the family	0	1	2
c)	Does homework without needing to be reminded	0	1	2
d)	Creative activities: art, acting, music, making things	0	1	2
e)	Likes to be involved in family activities	0	1	2
f)	Takes care of his appearance	0	1	2
g)	Good at school work	0	1	2
h)	Polite	0	1	2
i)	Good at sport	0	1	2
j)	Keeps his/her bedroom tidy	0	1	2
k)	Good with friends	0	1	2
l)	Well behaved	0	1	2

N3 Does [Name] have any other good points you particularly want to mention?

……..

Development and Well-being Assessment (Teacher Version)

Student's Name ..... Male / Female

Date of Birth ..... Form or Class .....

Teacher (form, year, subject etc.) .....

Signature ..... Today's Date .....

For each item, please mark the box. It would help us if you answered all items as best you can even if you are not absolutely certain or the item doesn't seem very relevant to this student. Please give your answers on the basis of the student's behaviour over the last six months or this school year.

Emotions

	Not True	Partly True	Certainly True
A1 Excessive worries	☐	☐	☐
A2 Marked tension or inability to relax	☐	☐	☐
A3 Excessive concern about his/her own abilities, (e.g. academic, sporting or social)	☐	☐	☐
A4 Particularly anxious about speaking to class or reading aloud	☐	☐	☐
A5 Reluctant to separate from family to come to school	☐	☐	☐
A6 Unhappy, sad or depressed	☐	☐	☐
A7 Has lost interest in carrying out usual activities	☐	☐	☐
A8 Feels worthless or inferior	☐	☐	☐
A9 Concentration affected by worries or misery	☐	☐	☐
A10 Other emotional difficulties (e.g. marked fears, panic attacks, obsessions or compulsions)	☐	☐	☐ *

*** A11 Please describe briefly:**

If you have ticked "Certainly True" to <u>any</u> of the questions A1 to A10, please complete the rest of this page. If not, go to the next page.

Do these difficulties	Not at all	A little	A medium amount	A great deal
A12 upset or distress him/her?	☐	☐	☐	☐
A13 interfere with his/her peer relationships?	☐	☐	☐	☐
A14 interfere with his/her classroom learning?	☐	☐	☐	☐
A15 put a burden on you or the class as a whole?	☐	☐	☐	☐

Attention, Activity and Impulsiveness

B1 When s/he is doing something in class that s/he enjoys and is good at, whether reading or drawing or making a model or whatever, how long does s/he typically stay on that task?

Less than 2 minutes	2-4 minutes	5-9 minutes	10-19 minutes	20 minutes or more
☐	☐	☐	☐	☐

	Not True	Partly True	Certainly True
B2 Makes careless mistakes	☐	☐	☐
B3 Fails to pay attention	☐	☐	☐
B4 Loses interest in what s/he is doing	☐	☐	☐
B5 Doesn't seem to listen	☐	☐	☐
B6 Fails to finish things s/he starts	☐	☐	☐
B7 Disorganised	☐	☐	☐
B8 Tries to avoid tasks that require thought	☐	☐	☐
B9 Loses things	☐	☐	☐
B10 Easily distracted	☐	☐	☐
B11 Forgetful	☐	☐	☐
B12 Fidgets	☐	☐	☐
B13 Can't stay seated when required to do so	☐	☐	☐
B14 Runs or climbs about when s/he shouldn't	☐	☐	☐
B15 Has difficulty playing quietly	☐	☐	☐
B16 Finds it hard to calm down when asked to do so	☐	☐	☐
B17 Blurts out answers before questions are finished	☐	☐	☐
B18 Hard for him/her to wait their turn	☐	☐	☐
B19 Interrupts, butts in on conversations or activities	☐	☐	☐
B20 Goes on talking if asked to stop	☐	☐	☐

If you have ticked "Certainly True" to <u>any</u> of the questions B2 to B20, please complete the rest of this page. If not, go to the next page.

Do these difficulties	Not at all	A little	A medium amount	A great deal
B21 upset or distress him/her?	☐	☐	☐	☐
B22 interfere with his/her peer relationships?	☐	☐	☐	☐
B23 interfere with his/her classroom learning?	☐	☐	☐	☐
B24 put a burden on you or the class as a whole?	☐	☐	☐	☐

Awkward and Troublesome Behaviour

	Not True	Partly True	Certainly True	
C1 Temper tantrums or hot tempers	☐	☐	☐	
C2 Argues a lot with adults	☐	☐	☐	
C3 Disobedient at school	☐	☐	☐	
C4 Deliberately does things to annoy others	☐	☐	☐	
C5 Blames others for his/her own mistakes	☐	☐	☐	
C6 Easily annoyed by others	☐	☐	☐	
C7 Angry and resentful	☐	☐	☐	
C8 Spiteful	☐	☐	☐	
C9 Tries to get his/her own back	☐	☐	☐	
C10 Seriously lies or cheats	☐	☐	☐	
C11 Starts fights	☐	☐	☐	
C12 Bullies others	☐	☐	☐	
C13 Plays truant	☐	☐	☐	
C14 Uses weapons when fighting	☐	☐	☐	
C15 Has been physically cruel, has really hurt someone	☐	☐	☐	
C16 Deliberately cruel to animals	☐	☐	☐	
C17 Sets fires deliberately	☐	☐	☐	
C18 Steals things	☐	☐	☐	*
C19 Vandalises property, or destroys things belonging to others	☐	☐	☐	*
C20 Shows unwanted sexualized behaviour towards others	☐	☐	☐	*
C21 Has been in trouble with the law	☐	☐	☐	*

*** C22 Please describe briefly:**

If you have ticked "Certainly True" to <u>any</u> of the questions C1 to C21, please complete the rest of this page. If not, go to the next page.

Do these behaviours	Not at all	A little	A medium amount	A great deal
C23 upset or distress him/her?	☐	☐	☐	☐
C24 interfere with his/her peer relationships?	☐	☐	☐	☐
C25 interfere with his/her classroom learning?	☐	☐	☐	☐
C26 put a burden on you or the class as a whole?	☐	☐	☐	☐

Other concerns

	Not True	Partly True	Certainly True
D1 Tics, twitches, involuntary grunts or noises	☐	☐	☐ *
D2 Diets to excess	☐	☐	☐ *

	No	Yes
D3 Do you have any other concerns about the child's psychological development?	☐	☐ *

*** D4 Please describe:**

D5 The rest of this page is for any additional comments about this child

Thank you very much for your help

Strengths and Difficulties Questionnaire P 4-17

For each item, please mark the box for Not True, Somewhat True or Certainly True. It would help us if you answered all items as best you can even if you are not absolutely certain or the item seems daft! Please give your answers on the basis of the child's behaviour over the last six months.

Child's Name ..... Male/Female

Date of Birth.....

	Not True	Somewhat True	Certainly True
Considerate of other people's feelings	☐	☐	☐
Restless, overactive, cannot stay still for long	☐	☐	☐
Often complains of headaches, stomach-aches or sickness	☐	☐	☐
Shares readily with other children (treats, toys, pencils etc.)	☐	☐	☐
Often has temper tantrums or hot tempers	☐	☐	☐
Rather solitary, tends to play alone	☐	☐	☐
Generally obedient, usually does what adults request	☐	☐	☐
Many worries, often seems worried	☐	☐	☐
Helpful if someone is hurt, upset or feeling ill	☐	☐	☐
Constantly fidgeting or squirming	☐	☐	☐
Has at least one good friend	☐	☐	☐
Often fights with other children or bullies them	☐	☐	☐
Often unhappy, down-hearted or tearful	☐	☐	☐
Generally liked by other children	☐	☐	☐
Easily distracted, concentration wanders	☐	☐	☐
Nervous or clingy in new situations, easily loses confidence	☐	☐	☐
Kind to younger children	☐	☐	☐
Often lies or cheats	☐	☐	☐
Picked on or bullied by other children	☐	☐	☐
Often volunteers to help others (parents, teachers, other children)	☐	☐	☐
Thinks things out before acting	☐	☐	☐
Steals from home, school or elsewhere	☐	☐	☐
Gets on better with adults than with other children	☐	☐	☐
Many fears, easily scared	☐	☐	☐
Sees tasks through to the end, good attention span	☐	☐	☐

Do you have any other comments or concerns?

Please turn over - there are a few more questions on the other side

Overall, do you think that your child has difficulties in one or more of the following areas: emotions, concentration, behaviour or being able to get on with other people?

No	Yes- minor difficulties	Yes- definite difficulties	Yes- severe difficulties
☐	☐	☐	☐

If you have answered "Yes", please answer the following questions about these difficulties:

- How long have these difficulties been present?

Less than a month	1-5 months	6-12 months	Over a year
☐	☐	☐	☐

- Do the difficulties upset or distress your child?

Not at all	Only a little	Quite a lot	A great deal
☐	☐	☐	☐

- Do the difficulties interfere with your child's everyday life in the following areas?

	Not at all	Only a little	Quite a lot	A great deal
HOME LIFE	☐	☐	☐	☐
FRIENDSHIPS	☐	☐	☐	☐
CLASSROOM LEARNING	☐	☐	☐	☐
LEISURE ACTIVITIES	☐	☐	☐	☐

- Do the difficulties put a burden on you or the family as a whole?

Not at all	Only a little	Quite a lot	A great deal
☐	☐	☐	☐

Signature ..... Date .....

Mother/Father/Other (please specify:)

Thank you very much for your help

Strengths and Difficulties Questionnaire T 4-17

For each item, please mark the box for Not True, Somewhat True or Certainly True. It would help us if you answered all items as best you can even if you are not absolutely certain or the item seems daft! Please give your answers on the basis of the child's behaviour over the last six months or this school year.

Child's Name ..... Male/Female

Date of Birth.....

	Not True	Somewhat True	Certainly True
Considerate of other people's feelings	☐	☐	☐
Restless, overactive, cannot stay still for long	☐	☐	☐
Often complains of headaches, stomach-aches or sickness	☐	☐	☐
Shares readily with other children (treats, toys, pencils etc.)	☐	☐	☐
Often has temper tantrums or hot tempers	☐	☐	☐
Rather solitary, tends to play alone	☐	☐	☐
Generally obedient, usually does what adults request	☐	☐	☐
Many worries, often seems worried	☐	☐	☐
Helpful if someone is hurt, upset or feeling ill	☐	☐	☐
Constantly fidgeting or squirming	☐	☐	☐
Has at least one good friend	☐	☐	☐
Often fights with other children or bullies them	☐	☐	☐
Often unhappy, down-hearted or tearful	☐	☐	☐
Generally liked by other children	☐	☐	☐
Easily distracted, concentration wanders	☐	☐	☐
Nervous or clingy in new situations, easily loses confidence	☐	☐	☐
Kind to younger children	☐	☐	☐
Often lies or cheats	☐	☐	☐
Picked on or bullied by other children	☐	☐	☐
Often volunteers to help others (parents, teachers, other children)	☐	☐	☐
Thinks things out before acting	☐	☐	☐
Steals from home, school or elsewhere	☐	☐	☐
Gets on better with adults than with other children	☐	☐	☐
Many fears, easily scared	☐	☐	☐
Sees tasks through to the end, good attention span	☐	☐	☐

Do you have any other comments or concerns?

Please turn over - there are a few more questions on the other side

Overall, do you think that this child has difficulties in one or more of the following areas: emotions, concentration, behaviour or being able to get on with other people?

No	Yes- minor difficulties	Yes- definite difficulties	Yes- severe difficulties
☐	☐	☐	☐

If you have answered "Yes", please answer the following questions about these difficulties:

- How long have these difficulties been present?

Less than a month	1-5 months	6-12 months	Over a year
☐	☐	☐	☐

- Do the difficulties upset or distress the child?

Not at all	Only a little	Quite a lot	A great deal
☐	☐	☐	☐

- Do the difficulties interfere with the child's everyday life in the following areas?

	Not at all	Only a little	Quite a lot	A great deal
PEER RELATIONSHIPS	☐	☐	☐	☐
CLASSROOM LEARNING	☐	☐	☐	☐

- Do the difficulties put a burden on you or the class as a whole?

Not at all	Only a little	Quite a lot	A great deal
☐	☐	☐	☐

Signature ..... Date .....

Class Teacher/Form Tutor/Head of Year/Other (please specify:)

Thank you very much for your help

The Adult Autism Spectrum Quotient (AQ)

Ages 16+

SPECIMEN, FOR RESEARCH USE ONLY.

Name:..... Sex:.....

Date of birth:..... Today's Date.....

How to fill out the questionnaire

Below are a list of statements. Please read each statement very carefully and rate how strongly you agree or disagree with it by circling your answer.

DO NOT MISS ANY STATEMENT OUT.

Examples

E1. I am willing to take risks.	definitely agree	slightly agree	slightly disagree	definitely disagree
E2. I like playing board games.	definitely agree	slightly agree	slightly disagree	definitely disagree
E3. I find learning to play musical instruments easy.	definitely agree	slightly agree	slightly disagree	definitely disagree
E4. I am fascinated by other cultures.	definitely agree	slightly agree	slightly disagree	definitely disagree

1. I prefer to do things with others rather than on my own.	definitely agree	slightly agree	slightly disagree	definitely disagree
2. I prefer to do things the same way over and over again.	definitely agree	slightly agree	slightly disagree	definitely disagree
3. If I try to imagine something, I find it very easy to create a picture in my mind.	definitely agree	slightly agree	slightly disagree	definitely disagree
4. I frequently get so strongly absorbed in one thing that I lose sight of other things.	definitely agree	slightly agree	slightly disagree	definitely disagree
5. I often notice small sounds when others do not.	definitely agree	slightly agree	slightly disagree	definitely disagree
6. I usually notice car number plates or similar strings of information.	definitely agree	slightly agree	slightly disagree	definitely disagree
7. Other people frequently tell me that what I've said is impolite, even though I think it is polite.	definitely agree	slightly agree	slightly disagree	definitely disagree
8. When I'm reading a story, I can easily imagine what the characters might look like.	definitely agree	slightly agree	slightly disagree	definitely disagree
9. I am fascinated by dates.	definitely agree	slightly agree	slightly disagree	definitely disagree
10. In a social group, I can easily keep track of several different people's conversations.	definitely agree	slightly agree	slightly disagree	definitely disagree
11. I find social situations easy.	definitely agree	slightly agree	slightly disagree	definitely disagree
12. I tend to notice details that others do not.	definitely agree	slightly agree	slightly disagree	definitely disagree
13. I would rather go to a library than a party.	definitely agree	slightly agree	slightly disagree	definitely disagree
14. I find making up stories easy.	definitely agree	slightly agree	slightly disagree	definitely disagree
15. I find myself drawn more strongly to people than to things.	definitely agree	slightly agree	slightly disagree	definitely disagree
16. I tend to have very strong interests which I get upset about if I can't pursue.	definitely agree	slightly agree	slightly disagree	definitely disagree
17. I enjoy social chit-chat.	definitely agree	slightly agree	slightly disagree	definitely disagree
18. When I talk, it isn't always easy for others to get a word in edgeways.	definitely agree	slightly agree	slightly disagree	definitely disagree

19. I am fascinated by numbers.	definitely agree	slightly agree	slightly disagree	definitely disagree
20. When I'm reading a story, I find it difficult to work out the characters' intentions.	definitely agree	slightly agree	slightly disagree	definitely disagree
21. I don't particularly enjoy reading fiction.	definitely agree	slightly agree	slightly disagree	definitely disagree
22. I find it hard to make new friends.	definitely agree	slightly agree	slightly disagree	definitely disagree
23. I notice patterns in things all the time.	definitely agree	slightly agree	slightly disagree	definitely disagree
24. I would rather go to the theatre than a museum.	definitely agree	slightly agree	slightly disagree	definitely disagree
25. It does not upset me if my daily routine is disturbed.	definitely agree	slightly agree	slightly disagree	definitely disagree
26. I frequently find that I don't know how to keep a conversation going.	definitely agree	slightly agree	slightly disagree	definitely disagree
27. I find it easy to "read between the lines" when someone is talking to me.	definitely agree	slightly agree	slightly disagree	definitely disagree
28. I usually concentrate more on the whole picture, rather than the small details.	definitely agree	slightly agree	slightly disagree	definitely disagree
29. I am not very good at remembering phone numbers.	definitely agree	slightly agree	slightly disagree	definitely disagree
30. I don't usually notice small changes in a situation, or a person's appearance.	definitely agree	slightly agree	slightly disagree	definitely disagree
31. I know how to tell if someone listening to me is getting bored.	definitely agree	slightly agree	slightly disagree	definitely disagree
32. I find it easy to do more than one thing at once.	definitely agree	slightly agree	slightly disagree	definitely disagree
33. When I talk on the phone, I'm not sure when it's my turn to speak.	definitely agree	slightly agree	slightly disagree	definitely disagree
34. I enjoy doing things spontaneously.	definitely agree	slightly agree	slightly disagree	definitely disagree
35. I am often the last to understand the point of a joke.	definitely agree	slightly agree	slightly disagree	definitely disagree
36. I find it easy to work out what someone is thinking or feeling just by looking at their face.	definitely agree	slightly agree	slightly disagree	definitely disagree
37. If there is an interruption, I can switch back to what I was doing very quickly.	definitely agree	slightly agree	slightly disagree	definitely disagree

38. I am good at social chit-chat.	definitely agree	slightly agree	slightly disagree	definitely disagree
39. People often tell me that I keep going on and on about the same thing.	definitely agree	slightly agree	slightly disagree	definitely disagree
40. When I was young, I used to enjoy playing games involving pretending with other children.	definitely agree	slightly agree	slightly disagree	definitely disagree
41. I like to collect information about categories of things (e.g. types of car, types of bird, types of train, types of plant, etc.).	definitely agree	slightly agree	slightly disagree	definitely disagree
42. I find it difficult to imagine what it would be like to be someone else.	definitely agree	slightly agree	slightly disagree	definitely disagree
43. I like to plan any activities I participate in carefully.	definitely agree	slightly agree	slightly disagree	definitely disagree
44. I enjoy social occasions.	definitely agree	slightly agree	slightly disagree	definitely disagree
45. I find it difficult to work out people's intentions.	definitely agree	slightly agree	slightly disagree	definitely disagree
46. New situations make me anxious.	definitely agree	slightly agree	slightly disagree	definitely disagree
47. I enjoy meeting new people.	definitely agree	slightly agree	slightly disagree	definitely disagree
48. I am a good diplomat.	definitely agree	slightly agree	slightly disagree	definitely disagree
49. I am not very good at remembering people's date of birth.	definitely agree	slightly agree	slightly disagree	definitely disagree
50. I find it very easy to play games with children that involve pretending.	definitely agree	slightly agree	slightly disagree	definitely disagree

Developed by:
The Autism Research Centre
University of Cambridge

The Adult Autism Spectrum Quotient (AQ)

Ages 16+: Scoring Key

For full details, please see:

S. Baron-Cohen, S. Wheelwright, R. Skinner, J. Martin and E. Clubley, (2001)
The Autism Spectrum Quotient (AQ) : Evidence from Asperger Syndrome/High Functioning Autism, Males and Females, Scientists and Mathematicians
Journal of Autism and Developmental Disorders 31:5-17

Responses that score 1 point are marked. Other responses score 0. For total score, sum all items.

		definitely agree	slightly agree	slightly disagree	definitely disagree
1.	I prefer to do things with others rather than on my own.			1	1
2.	I prefer to do things the same way over and over again.	1	1		
3.	If I try to imagine something, I find it very easy to create a picture in my mind.			1	1
4.	I frequently get so strongly absorbed in one thing that I lose sight of other things.	1	1		
5.	I often notice small sounds when others do not.	1	1		
6.	I usually notice car number plates or similar strings of information.	1	1		
7.	Other people frequently tell me that what I've said is impolite, even though I think it is polite.	1	1		
8.	When I'm reading a story, I can easily imagine what the characters might look like.			1	1
9.	I am fascinated by dates.	1	1		
10.	In a social group, I can easily keep track of several different people's conversations.			1	1
11.	I find social situations easy.			1	1
12.	I tend to notice details that others do not.	1	1		

		definitely agree	slightly agree	slightly disagree	definitely disagree
13.	I would rather go to a library than a party.	1	1		
14.	I find making up stories easy.			1	1
15.	I find myself drawn more strongly to people than to things.			1	1
16.	I tend to have very strong interests which I get upset about if I can't pursue.	1	1		
17.	I enjoy social chit-chat.			1	1
18.	When I talk, it isn't always easy for others to get a word in edgeways.	1	1		
19.	I am fascinated by numbers.	1	1		
20.	When I'm reading a story, I find it difficult to work out the characters' intentions.	1	1		
21.	I don't particularly enjoy reading fiction.	1	1		
22.	I find it hard to make new friends.	1	1		
23.	I notice patterns in things all the time.	1	1		
24.	I would rather go to the theatre than a museum.			1	1
25.	It does not upset me if my daily routine is disturbed.			1	1
26.	I frequently find that I don't know how to keep a conversation going.	1	1		
27.	I find it easy to "read between the lines" when someone is talking to me.			1	1
28.	I usually concentrate more on the whole picture, rather than the small details.			1	1
29.	I am not very good at remembering phone numbers.			1	1
30.	I don't usually notice small changes in a situation, or a person's appearance.			1	1
31.	I know how to tell if someone listening to me is getting bored.			1	1
32.	I find it easy to do more than one thing at once.			1	1

		definitely agree	slightly agree	slightly disagree	definitely disagree
33.	When I talk on the phone, I'm not sure when it's my turn to speak.	1	1		
34.	I enjoy doing things spontaneously.			1	1
35.	I am often the last to understand the point of a joke.	1	1		
36.	I find it easy to work out what someone is thinking or feeling just by looking at their face.			1	1
37.	If there is an interruption, I can switch back to what I was doing very quickly.			1	1
38.	I am good at social chit-chat.			1	1
39.	People often tell me that I keep going on and on about the same thing.	1	1		
40.	When I was young, I used to enjoy playing games involving pretending with other children.			1	1
41.	I like to collect information about categories of things (e.g. types of car, types of bird, types of train, types of plant, etc.).	1	1		
42.	I find it difficult to imagine what it would be like to be someone else.	1	1		
43.	I like to plan any activities I participate in carefully.	1	1		
44.	I enjoy social occasions.			1	1
45.	I find it difficult to work out people's intentions.	1	1		
46.	New situations make me anxious.	1	1		
47.	I enjoy meeting new people.			1	1
48.	I am a good diplomat.			1	1
49.	I am not very good at remembering people's date of birth.			1	1
50.	I find it very easy to play games with children that involve pretending.			1	1

Hospital Anxiety and Depression Scale (HADS)

Instructions: Doctors are aware that emotions play an important part in most illnesses. If your doctor knows about these feelings he or she will be able to help you more. This questionnaire is designed to help your doctor know how you feel. Read each item and circle the reply which comes closest to how you have been feeling in the past week. Don't take too long over your replies: your immediate reaction to each item will probably be more accurate than a long thought out response.

I feel tense or 'wound up':	**A**
Most of the time	3
A lot of the time	2
Time to time, occasionally	1
Not at all	0

I feel as if I am slowed down:	**D**
Nearly all of the time	3
Very often	2
Sometimes	1
Not at all	0

I still enjoy the things I used to enjoy:	**D**
Definitely as much	0
Not quite so much	1
Only a little	2
Not at all	3

I get a sort of frightened feeling like 'butterflies in the stomach':	**A**
Not at all	0
Occasionally	1
Quite often	2
Very often	3

I get a sort of frightened feeling like something awful is about to happen:	**A**
Very definitely and quite badly	3
Yes, but not too badly	2
A little, but it doesn't worry me	1
Not at all	0

I have lost interest in my appearance:	**D**
Definitely	3
I don't take as much care as I should	2
I may not take quite as much care	1
I take just as much care as ever	0

I can laugh and see the funny side of things:	**D**
As much as I always could	0
Not quite so much now	1
Definitely not so much now	2
Not at all	3

I feel restless as if I have to be on the move:	**A**
Very much indeed	3
Quite a lot	2
Not very much	1
Not at all	0

Worrying thoughts go through my mind:	**A**
A great deal of the time	3
A lot of the time	2
From time to time but not too often	1
Only occasionally	0

I look forward with enjoyment to things:	**D**
A much as I ever did	0
Rather less than I used to	1
Definitely less than I used to	3
Hardly at all	2

I feel cheerful:	**D**
Not at all	3
Not often	2
Sometimes	1
Most of the time	0

I get sudden feelings of panic:	**A**
Very often indeed	3
Quite often	2
Not very often	1
Not at all	0

I can sit at ease and feel relaxed:	**A**
Definitely	0
Usually	1
Not often	2
Not at all	3

I can enjoy a good book or radio or TV programme:	**D**
Often	0
Sometimes	1
Not often	2
Very seldom	3

Questions relating to anxiety are indicated by an 'A' while those relating to depression are shown by a 'D'. Scores of 0-7 in respective subscales are considered normal, with 8-10 borderline and 11 or over indicating clinical 'caseness'

Cambridge Behavioural Inventory Revised (CBI-R)

For the Carer

Your Name:_____ Today's date:___/___/___
Patient's name: _____Relationship to the patient_____

We would like to ask you a number of questions about various changes in the patient's behaviour that you may have noticed. It is important that we obtain your view as it will help us in our assessment.
Please read the description of each problem carefully. Then circle the number under the heading "Frequency" that best describes the occurrence of the behavioural change.
Some of the everyday skill questions may not apply, if for instance the person you care for has never done the shopping. Please enter N/A (not applicable).

All questions apply to the patient's behaviour OVER THE PAST MONTH.

0 Never	1 a few times per month	2 a few times per week	3 daily	4 constantly

	FREQUENCY				
Memory and Orientation					
Has poor day-to-day memory (e.g. about conversations, trips etc.)	0	1	2	3	4
Asks the same questions over and over again	0	1	2	3	4
Loses or misplaces things	0	1	2	3	4
Forgets the names of familiar people	0	1	2	3	4
Forgets the names of objects and things	0	1	2	3	4
Shows poor concentration when reading or watching television	0	1	2	3	4
Forgets what day it is	0	1	2	3	4
Becomes confused or muddled in unusual surroundings	0	1	2	3	4
Everyday Skills					
Has difficulties using electrical appliances (e.g. TV, radio, cooker, washing machine)	0	1	2	3	4
Has difficulties writing (letters, Christmas cards, lists etc.)	0	1	2	3	4
Has difficulties using the telephone	0	1	2	3	4
Has difficulties making a hot drink (e.g. tea/coffee)	0	1	2	3	4
Has problems handling money or paying bills	0	1	2	3	4
Self Care					
Has difficulties grooming self (e.g. shaving or putting on make-up)	0	1	2	3	4
Has difficulties dressing self	0	1	2	3	4
Has problems feeding self without assistance	0	1	2	3	4
Has problems bathing or showering self	0	1	2	3	4
Abnormal Behaviour					
Finds humour or laughs at things others do not find funny	0	1	2	3	4
Has temper outbursts	0	1	2	3	4
Is uncooperative when asked to do something	0	1	2	3	4
Shows socially embarrassing behaviour	0	1	2	3	4
Makes tactless or suggestive remarks	0	1	2	3	4
Acts impulsively without thinking	0	1	2	3	4

Cambridge Behavioural Inventory Revised (CBI-R)

0 Never	1 a few times per month	2 a few times per week	3 daily	4 constantly

Mood						
Cries	0	1	2	3	4	
Appears sad or depressed	0	1	2	3	4	
Is very restless or agitated	0	1	2	3	4	
Is very irritable	0	1	2	3	4	
Beliefs						
Sees things that are not really there (visual hallucinations)	0	1	2	3	4	
Hears voices that are not really there (auditory hallucinations)	0	1	2	3	4	
Has odd or bizarre ideas that cannot be true	0	1	2	3	4	
Eating Habits						
Prefers sweet foods more than before	0	1	2	3	4	
Wants to eat the same foods repeatedly	0	1	2	3	4	
Her/his appetite is greater, s/he eats more than before	0	1	2	3	4	
Table manners are declining e.g. stuffing food into mouth	0	1	2	3	4	
Sleep						
Sleep is disturbed at night	0	1	2	3	4	
Sleeps more by day than before (cat naps etc.)	0	1	2	3	4	
Stereotypic and Motor Behaviours						
Is rigid and fixed in her/his ideas and opinions	0	1	2	3	4	
Develops routines from which s/he can not easily be discouraged e.g. wanting to eat or go for walks at fixed times	0	1	2	3	4	
Clock watches or appears pre-occupied with time	0	1	2	3	4	
Repeatedly uses the same expression or catch phrase	0	1	2	3	4	
Motivation						
Shows less enthusiasm for his or her usual interests	0	1	2	3	4	
Shows little interest in doing new things	0	1	2	3	4	
Fails to maintain motivation to keep in contact with friends or family	0	1	2	3	4	
Appears indifferent to the worries and concerns of family members	0	1	2	3	4	
Shows reduced affection	0	1	2	3	4	

Any other comments:

Thank you for your time.

ADDENBROOKE'S COGNITIVE EXAMINATION - ACE-R

Final Revised Version A (May 2004) - Australian Version

Name :
Date of birth :
Hospital no. :

Addressograph

Date of testing: ..... /..... /.....
Tester's name: .....
Age at leaving full-time education: .....
Occupation: .....
Handedness: .....

ORIENTATION

[Score 0-5]

➢ Ask: What is the | Day | Date | Month | Year | Season

➢ Ask: Which | Building | Floor | Town | State | Country

[Score 0-5]

REGISTRATION

[Score 0-3]

➢ Tell: 'I'm going to give you three words and I'd like you to repeat after me: lemon, key and ball'. After subject repeats, say 'Try to remember them because I'm going to ask you later'. Score only the first trial (repeat 3 times if necessary).

Register number of trials .....

ATTENTION & CONCENTRATION

[Score 0-5]

(for the best performed task)

➢ Ask the subject: ' could you take 7 away from a 100? After the subject responds, ask him or her to take away another 7 to a total of 5 subtractions. If subject make a mistake, carry on and check the subsequent answer (i.e. 93, 84, 77, 70, 63 -score 4)

Stop after five subtractions (93, 86, 79, 72, 65). ....

➢ Ask: 'could you please spell **WORLD** for me? Then ask him/her to spell it backwards:

....

MEMORY - Recall

[Score 0-3]

➢ Ask: 'Which 3 words did I ask you to repeat and remember?'

....

MEMORY - Anterograde Memory

[Score 0-7]

➢ Tell: ' I'm going to give you a name and address and I'd like you to repeat after me. We'll be doing that 3 times, so you have a chance to learn it. I'll be asking you later'

Score only the third trial

	1st Trial	2nd Trial	3rd Trial
Harry Barnes	....	....	....
73 Market Street	....	....	....
Rockhampton	.....	.....	.....
Queensland	.....	.....	.....

MEMORY - Retrograde Memory

[Score 0 -4]

➢ Name of current Prime Minister .....
➢ Name of the Premier of New South Wales .....
➢ Name of the USA president .....
➢ Name of the USA president who was assassinated in the 1960s .....

ATTENTION&ORIENTATION MEMORY

VERBAL FLUENCY - Letter 'P' and animals

➢ **Letters**

Say: 'I'm going to give you a letter of the alphabet and I'd like you to generate as many words as you can beginning with that letter, but not names of people or places. Are you ready? You've got a minute and the letter is P'

[Score 0 - 7] ☐

				>17	7
				14-17	6
				11-13	5
				8-10	4
				6-7	3
				4-5	2
				3-4	1
				<3	0
				total	correct

➢ **Animals**

Say: 'Now can you name as many animals as possible, beginning with any letter?

[Score 0 - 7] ☐

				>21	7
				17-21	6
				14-16	5
				11-13	4
				9-10	3
				7-8	2
				5-6	1
				<5	0
				total	correct

FLUENCY

LANGUAGE - Comprehension

➢ Show written instruction:

[Score 0-1] ☐ ☐

Close your eyes

➢ 3 stage command:
'Take the paper in your right hand. Fold the paper in half. Put the paper on the floor'

[Score 0-3] ☐ ☐

LANGUAGE - Writing

➢ Ask the subject to make up a sentence and write it in the space below:
Score 1 if sentence contains a subject and a verb (see guide for examples)

[Score 0-1] ☐ ☐

LANGUAGE

L A N G U A G E - Repetition

[Score 0-2]

➢ Ask the subject to repeat:**' hippopotamus'; 'eccentricity; 'unintelligible'; 'statistician'**
Score 2 if all correct; 1 if 3 correct; 0 if 2 or less.

[Score 0-1]

➢ Ask the subject to repeat: **'Above, beyond and below'**

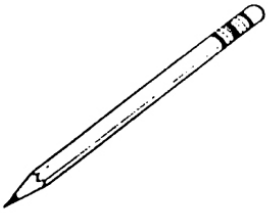
[Score 0-1]

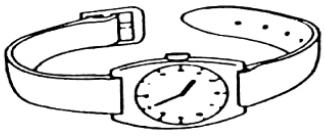
➢ Ask the subject to repeat: **'No ifs, ands or buts'**

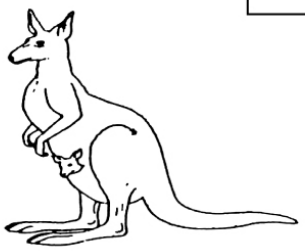
L A N G U A G E - Naming

➢ Ask the subject to name the following pictures:

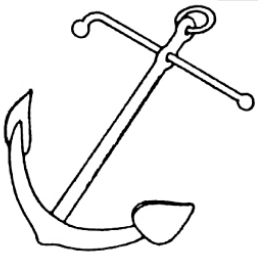
[Score 0-2]
pencil +
watch

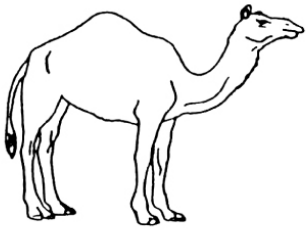


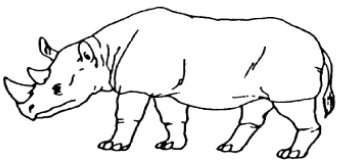


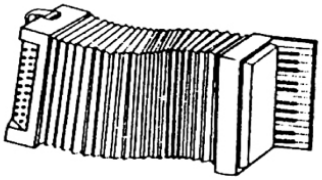


[Score 0-10]









L A N G U A G E - Comprehension

[Score 0-4]

➢ Using the pictures above, ask the subject to:

- Point to the one which is associated with the monarchy _____
- Point to the one which is a marsupial _____
- Point to the one which is found in the Antarctic _____
- Point to the one which has a nautical connection _____

LANGUAGE - Reading

➢ Ask the subject to read the following words: [Score 1 only if all correct]

[Score 0-1] ☐

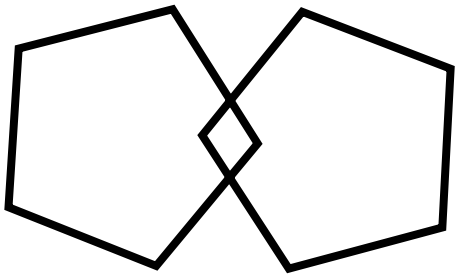
sew
pint
soot
dough
height

LANGUAGE

VISUOSPATIAL ABILITIES

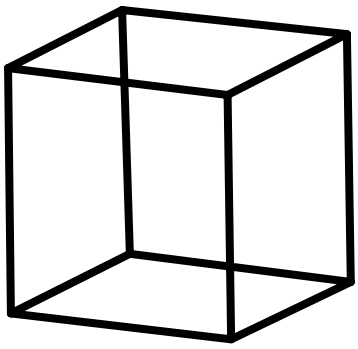
➢ Overlapping pentagons: Ask the subject to copy this diagram:

[Score 0-1] ☐ ☐



➢ Wire cube : Ask the subject to copy this drawing (for scoring, see instructions guide)

[Score 0-2] ☐



➢ Clock: Ask the subject to draw a clock face with numbers and the hands at ten past five. (for scoring see instruction guide: circle = 1, numbers = 2, hands = 2 if all correct)

[Score 0-5] ☐

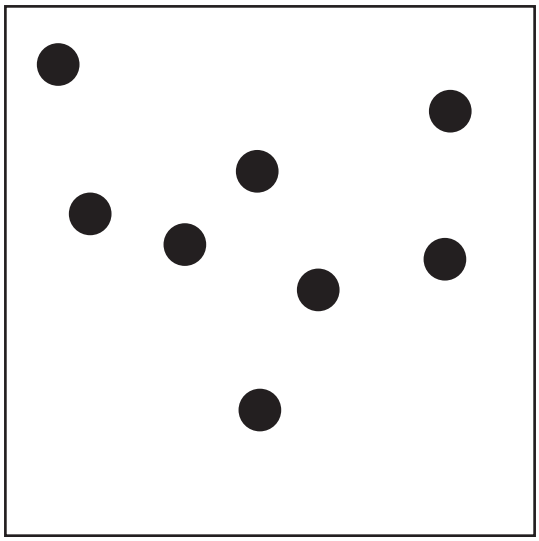
VISUOSPATIAL

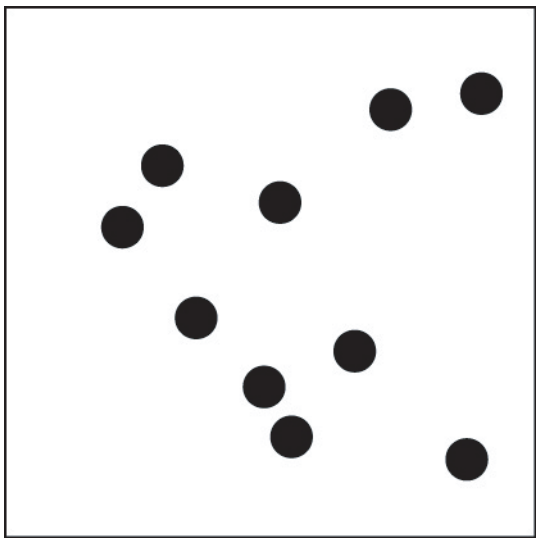
PERCEPTUAL ABILITIES

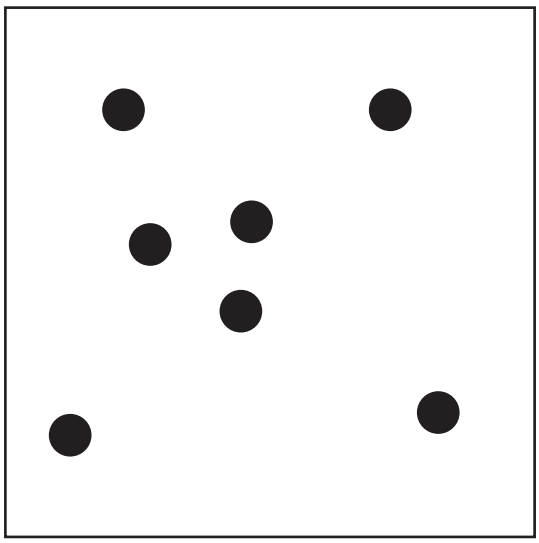
VISUOSPATIAL

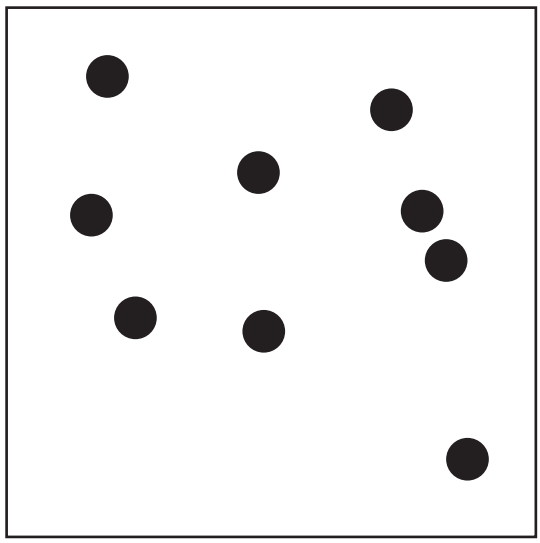
➢ Ask the subject to count the dots without pointing them

[Score 0-4]





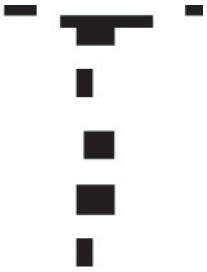




PERCEPTUAL ABILITIES

➢ Ask the subject to identify the letters

[Score 0-4] ☐



RECALL

➢ Ask "Now tell me what you remember of that name and address we were repeating at the beginning'"

Harry Barnes
73 Market Street
Rockhampton
Queensland

....
....
.....
.....

[Score 0-7] ☐

RECOGNITION

➢ This test should be done if subject failed to recall one or more items. If all items were recalled, skip the test and score 5. If only part is recalled start by ticking items recalled in the shadowed column on the right hand side. Then test not recalled items by telling "ok, I'll give you some hints: was the name X, Y or Z?" and so on. Each recognised item scores one point which is added to the point gained by recalling.

[Score 0-5] ☐

Jerry Barne		Harry Barnes		Harry Bradford		recalled	
37		73		76		recalled	
Market Road		Martin Street		Market Street		recalled	
Margate		Rockhampton		Cairns		recalled	
Queensland		New South Wales		Victoria		recalled	

General Scores

MMSE	/30
ACE-R	/100

Subscores

Attention and Orientation	/18
Memory	/26
Fluency	/14
Language	/26
Visuospatial	/16

VISUOSPATIAL MEMORY SCORE